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<p>(54) Title: MANIPULATION OF CELLULOSE AND/OR <math>\beta</math>-1,4-GLUCAN</p> <p>(57) Abstract</p> <p>The present invention relates generally to isolated genes which encode polypeptides involved in cellulose biosynthesis in plants and transgenic plants expressing same in sense or antisense orientation, or as ribozymes, co-suppression or gene-targeting molecules. More particularly, the present invention is directed to a nucleic acid molecule isolated from <i>Arabidopsis thaliana</i>, <i>Oryza sativa</i>, wheat, barley, maize, <i>Brassica</i> spp., <i>Gossypium hirsutum</i> and <i>Eucalyptus</i> spp. which encode an enzyme which is important in cellulose biosynthesis, in particular the cellulose synthase enzyme and homologues, analogues and derivatives thereof and uses of same in the production of transgenic plants expressing altered cellulose biosynthetic properties.</p>			

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- 1 -

## "MANIPULATION OF CELLULOSE AND/OR $\beta$ -1,4-GLUCAN"

The present invention relates generally to isolated genes which encode polypeptides involved in cellulose biosynthesis and transgenic organisms expressing same in sense or antisense orientation, or as ribozymes, co-suppression or gene-targeting molecules. More particularly, the present invention is directed to a nucleic acid molecule isolated from *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* ssp., *Gossypium hirsutum* and *Eucalyptus* ssp. which encode an enzyme which is important in cellulose biosynthesis, in particular the cellulose synthase enzyme and homologues, analogues and derivatives thereof and uses of same in the production of transgenic plants expressing altered cellulose biosynthetic properties.

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description. Sequence identity numbers (SEQ ID Nos.) for the nucleotide and amino acid sequences referred to in the specification are defined after the bibliography.

Throughout the specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Cellulose, the world's most abundant biopolymer, is the most characteristic component of plant cell walls in so far as it forms much of the structural framework of the cell wall. Cellulose is comprised of crystalline  $\beta$ -1,4-glucan microfibrils. The crystalline microfibrils are extremely strong and resist enzymic and mechanical degradation, an important factor in determining the nutritional quantity, digestibility and palatability of animal and human foodstuffs. As cellulose is also the dominant structural component of industrially-important plant fibres, such as cotton, flax, hemp, jute and the timber crops such as *Eucalyptus* ssp. and *Pinus* ssp., amongst others, there is considerable economic benefit to be derived from the

- 2 -

manipulation of cellulose content and/or quantity in plants. In particular, the production of food and fibre crops with altered cellulose content are highly desirable objectives.

The synthesis of cellulose involves the  $\beta$ -1,4-linkage of glucose monomers, in the form of a 5 nucleoside diphosphoglucose such as UDP-glucose, to a pre-existing cellulose chain, catalysed by the enzyme cellulose synthase.

Several attempts to identify the components of the functional cellulose synthase in plants have failed, because levels of  $\beta$ -1,4-glucan or crystalline cellulose produced in such assays have 10 hitherto been too low to permit enzyme purification for protein sequence determination. Insufficient homology between bacterial  $\beta$ -1,4-glucan synthase genes and plant cellulose synthase genes has also prevented the use of hybridisation as an approach to isolating the plant homologues of bacterial  $\beta$ -1,4-glucan (cellulose) synthases.

15 Furthermore, it has not been possible to demonstrate that the cellulose synthase enzyme from plants is the same as, or functionally related to, other purified and characterised enzymes involved in polysaccharide biosynthesis. As a consequence, the cellulose synthase enzyme has not been isolated from plants and, until the present invention, no nucleic acid molecule has been characterised which functionally-encodes a plant cellulose synthase enzyme.

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In work leading up to the present invention, the inventors have generated several novel mutant *Arabidopsis thaliana* plants which are defective in cellulose biosynthesis. The inventors have further isolated a cellulose synthase gene designated *RSW1*, which is involved 25 in cellulose biosynthesis in *Arabidopsis thaliana*, and homologous sequences in *Oryza sativa*, wheat, barley, maize, *Brassica* ssp., *Gossypium hirsutum* and *Eucalyptus* ssp. The isolated nucleic acid molecules of the present invention provide the means by which cellulose content and structure may be modified in plants to produce a range of useful fibres suitable for specific industrial purposes, for example increased decay resistance of timber and altered digestibility of foodstuffs, amongst others.

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- 3 -

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes, or is complementary to a sequence which encodes a polypeptide of the cellulose biosynthetic pathway or a functional homologue, analogue or derivative thereof.

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The nucleic acid molecule of the invention may be derived from a prokaryotic source or a eukaryotic source.

Those skilled in the art will be aware that cellulose production requires not only the presence  
10 of a catalytic subunit, but also its activation and organisation into arrays which favour the crystallization of glucan chains. This organisation is radically different between bacteria, which possess linear arrays, and higher plants, which possess hexameric clusters or "rosettes", of glucan chains. The correct organisation and activation of the bacterial enzyme may require many factors which are either not known, or alternatively, not known to be  
15 present in plant cells, for example specific membrane lipids to impart an active conformation on the enzyme complex or protein, or the bacterial c-di-GMP activation system.  
Accordingly, the use of a plant-derived sequence in eukaryotic cells such as plants provides significant advantages compared to the use of bacterially-derived sequences.

20 Accordingly, the present invention does not extend to known genes encoding the catalytic subunit of *Agrobacterium tumefaciens* or *Acetobacter xylinum* or *Acetobacter pasteurianus* cellulose synthase, or the use of such known bacterial genes and polypeptides to manipulate cellulose.

25 Preferably, the subject nucleic acid molecule is derived from an eukaryotic organism.

In a more preferred embodiment of the invention, the isolated nucleic acid molecule of the invention encodes a plant cellulose synthase or a catalytic subunit thereof, or a homologue, analogue or derivative thereof.

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- 4 -

More preferably, the isolated nucleic acid molecule encodes a plant cellulose synthase polypeptide which is associated with the primary cell wall of a plant cell. In an alternative preferred embodiment, the nucleic acid molecule of the invention encodes a plant cellulose synthase or catalytic subunit thereof which is normally associated with the secondary cell wall 5 of a plant cell.

In a more preferred embodiment, the nucleic acid molecule of the invention is a cDNA molecule, genomic clone, mRNA molecule or a synthetic oligonucleotide molecule.

10 In a particularly preferred embodiment, the present invention provides an isolated nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes the *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* ssp., *Brassica* ssp. wheat, barley or maize cellulose synthase enzyme or a catalytic subunit thereof or a polypeptide component, homologue, analogue or derivative thereof.

15

As exemplified herein, the present inventors have identified cellulose biosynthesis genes in maize, wheat, barley, rice, cotton, *Brassica* ssp. and *Eucalyptus* ssp., in addition to the specific *Arabidopsis thaliana RSW1* gene sequence which has been shown to be particularly useful for altering cellulose and/or  $\beta$ -1,4-glucan and/or starch levels in cells.

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Hereinafter the term "polypeptide of the cellulose biosynthetic pathway" or similar term shall be taken to refer to a polypeptide or a protein or a part, homologue, analogue or derivative thereof which is involved in one or more of the biosynthetic steps leading to the production of cellulose or any related  $\beta$ -1,4-glucan polymer in plants. In the present context, a 25 polypeptide of the cellulose biosynthetic pathway shall also be taken to include both an active enzyme which contributes to the biosynthesis of cellulose or any related  $\beta$ -1,4-glucan polymer in plants and to a polypeptide component of such an enzyme. As used herein, a polypeptide of the cellulose biosynthetic pathway thus includes cellulose synthase. Those skilled in the art will be aware of other cellulose biosynthetic pathway polypeptides in plants.

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- 5 -

The term "related  $\beta$ -1,4-glucan polymer" shall be taken to include any carbohydrate molecule comprised of a primary structure of  $\beta$ -1,4-linked glucose monomers similar to the structure of the components of the cellulose microfibril, wherein the relative arrangement or relative configuration of the glucan chains may differ from their relative configuration in microfibrils 5 of cellulose. As used herein, a related  $\beta$ -1,4-glucan polymer includes those  $\beta$ -1,4-glucan polymers wherein individual  $\beta$ -1,4-glucan microfibrils are arranged in an anti-parallel or some other relative configuration not found in a cellulose molecule of plants and those non-crystalline  $\beta$ -1,4-glucans described as lacking the resistance to extraction and degradation that characterise cellulose microfibrils.

10

The term "cellulose synthase" shall be taken to refer to a polypeptide which is required to catalyse a  $\beta$ -1,4-glucan linkage to a cellulose microfibril.

Reference herein to "gene" is to be taken in its broadest context and includes:

15

- (i) a classical genomic gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e. introns, 5'- and 3'- untranslated sequences); or
- (ii) mRNA or cDNA corresponding to the coding regions (i.e. exons) and 5'- and 3'- untranslated sequences of the gene.

20

The term "gene" is also used to describe synthetic or fusion molecules encoding all or part of a functional product.

25

In the present context, the term "cellulose gene" or "cellulose genetic sequence" or similar term shall be taken to refer to any gene as hereinbefore defined which encodes a polypeptide of the cellulose biosynthetic pathway and includes a cellulose synthase gene.

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The term "cellulose synthase gene" shall be taken to refer to any cellulose gene which specifically encodes a polypeptide which is a component of a functional enzyme having cellulose synthase activity i.e. an enzyme which catalyses a  $\beta$ -1,4-glucan linkage to a

cellulose microfibril.

Preferred cellulose genes may be derived from a naturally-occurring cellulose gene by standard recombinant techniques. Generally, a cellulose gene may be subjected to 5 mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the cellulose synthase gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although 10 random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. 15 Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity.

As used herein, the term "derived from" shall be taken to indicate that a particular integer or group of integers has originated from the species specified, but has not necessarily been 20 obtained directly from the specified source.

For the present purpose, "homologues" of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the 25 occurrence within said sequence, of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

"Analogue" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present 30 invention or its complementary nucleotide sequence, notwithstanding the occurrence of any

non-nucleotide constituents not normally present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

5 "Derivatives" of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include  
10 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are  
15 characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

The present invention extends to the isolated nucleic acid molecule when integrated into the  
20 genome of a cell as an addition to the endogenous cellular complement of cellulose synthase genes. The said integrated nucleic acid molecule may, or may not, contain promoter sequences to regulate expression of the subject genetic sequence.

The isolated nucleic acid molecule of the present invention may be introduced into and  
25 expressed in any cell, for example a plant cell, fungal cell, insect cell, animal cell, yeast cell or bacterial cell. Those skilled in the art will be aware of any modifications which are required to the codon usage or promoter sequences or other regulatory sequences, in order for expression to occur in such cells.

30 Another aspect of the present invention is directed to a nucleic acid molecule which comprises

a sequence of nucleotides corresponding or complementary to any one or more of the sequences set forth in SEQ ID Nos:1, 3, 4, 5, 7, 9, 11, or 13, or having at least about 40%, more preferably at least about 55%, still more preferably at least about 65%, yet still more preferably at least about 75-80% and even still more preferably at least about 85-95%

5 nucleotide similarity to all, or a part thereof.

According to this aspect of the invention, said nucleic acid molecule encodes, or is complementary to a nucleotide sequence encoding, a polypeptide of the cellulose biosynthetic pathway in a plant or a homologue, analogue or derivative thereof.

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Preferably, a nucleic acid molecule which is at least 40% related to any one or more of the sequences set forth in SEQ ID Nos:1, 3, 4, 5, 7, 9, 11, or 13 comprises a nucleotide sequence which encodes or is complementary to a sequence which encodes a plant cellulose synthase, more preferably a cellulose synthase which is associated with the primary or the  
15 secondary plant cell wall of the species from which it has been derived.

Furthermore, the nucleic acid molecule according to this aspect of the invention may be derived from a monocotyledonous or dicotyledonous plant species. In a particularly preferred embodiment, the nucleic acid molecule is derived from *Arabidopsis thaliana*, *Oryza sativa*,  
20 wheat, barley, maize, *Brassica* ssp., *Gossypium hirsutum* (cotton) or *Eucalyptus* ssp., amongst others.

For the purposes of nomenclature, the nucleotide sequence shown in SEQ ID NO:1 relates to a cellulose gene as hereinbefore defined which comprises a cDNA sequence designated  
25 T20782 and which is derived from *Arabidopsis thaliana*. The amino acid sequence set forth in SEQ ID NO:2 relates to the polypeptide encoded by T20782.

The nucleotide sequence set forth in SEQ ID NO:3 relates to the nucleotide sequence of the complete *Arabidopsis thaliana* genomic gene *RSW1*, including both intron and exon  
30 sequences. The nucleotide sequence of SEQ ID NO:3 comprises exons 1-14 of the genomic

gene and includes 2295bp of 5'-untranslated sequences, of which approximately the first 1.9kb comprises *RSW1* promoter sequence (there is a putative TATA box motif at positions 1843-1850 of SEQ ID NO:3). The nucleotide sequence set forth in SEQ ID NO:3 is derived from the cosmid clone 23H12. This sequence is also the genomic gene equivalent of SEQ ID Nos:1 and 5.

The nucleotide sequence set forth in SEQ ID NO:4 relates to the partial nucleotide sequence of a genomic gene variant of *RSW1*, derived from cosmid clone 12C4. The nucleotide sequence of SEQ ID NO:4 comprises exon sequence 1-11 and part of exon 12 of the genomic gene sequence and includes 862bp of 5'-untranslated sequences, of which approximately 700 nucleotides comprise *RSW1* promoter sequences (there is a putative TATA box motif at positions 668-673 of SEQ ID NO:4). The genomic gene sequence set forth in SEQ ID NO:4 is the equivalent of the cDNA sequence set forth in SEQ ID NO:7 (i.e. cDNA clone *Ath-A*).

15 The nucleotide sequence shown in SEQ ID NO:5 relates to a cellulose gene as hereinbefore defined which comprises a cDNA equivalent of the *Arabidopsis thaliana RSW1* gene set forth in SEQ ID NO:3. The amino acid sequence set forth in SEQ ID NO:6 relates to the polypeptide encoded by the wild-type *RSW1* gene sequences set forth in SEQ ID Nos:3 and 5.

20

The nucleotide sequence shown in SEQ ID NO:7 relates to a cellulose gene as hereinbefore defined which comprises a cDNA equivalent of the *Arabidopsis thaliana RSW1* gene set forth in SEQ ID NO:4. The nucleotide sequence is a variant of the nucleotide sequences set forth in SEQ ID Nos:3 and 5. The amino acid sequence set forth in SEQ ID NO:8 relates to the 25 polypeptide encoded by the wild-type *RSW1* gene sequences set forth in SEQ ID Nos:4 and 6.

The nucleotide sequence shown in SEQ ID NO:9 relates to a cellulose gene as hereinbefore defined which comprises a further wild-type variant of the *Arabidopsis thaliana RSW1* gene 30 set forth in SEQ ID Nos:3 and 5. The nucleotide sequence variant is designated *Ath-B*. The

- 10 -

amino acid sequence set forth in SEQ ID NO:10 relates to the polypeptide encoded by the wild-type *RSW1* gene sequence set forth in SEQ ID No:9.

The nucleotide sequence shown in SEQ ID NO:11 relates to a cellulose gene as hereinbefore defined which comprises a cDNA equivalent of the *Arabidopsis thaliana rsw1* gene. The *rsw1* gene is a mutant cellulose gene which produces a radial root swelling phenotype as described by Baskin *et al* (1992). The present inventors have shown herein that the *rsw1* gene also produces reduced inflorescence length, reduced fertility, misshapen epidermal cells, reduced cellulose content and the accumulation of non-crystalline  $\beta$ -1,4-glucan, amongst others, when expressed in plant cells. The *rsw1* nucleotide sequence is a further variant of the nucleotide sequences set forth in SEQ ID Nos:3 and 5. The amino acid sequence set forth in SEQ ID NO:12 relates to the *rsw1* polypeptide encoded by the mutant *rsw1* gene sequence set forth in SEQ ID No:11.

15 The nucleotide sequence shown in SEQ ID NO:13 relates to a cellulose gene as hereinbefore defined which comprises a cDNA equivalent of the *Oryza sativa RSW1* or *RSW1*-like gene. The nucleotide sequence is closely-related to the *Arabidopsis thaliana RSW1* and *rsw1* nucleotide sequences set forth herein (SEQ ID Nos:1, 3, 4, 5, 7, 9 and 11). The amino acid sequence set forth in SEQ ID NO:14 relates to the polypeptide encoded by the *RSW1* or  
20 *RSW1*-like gene sequences set forth in SEQ ID No:13.

Those skilled in the art will be aware of procedures for the isolation of further cellulose genes to those specifically described herein, for example further cDNA sequences and genomic gene equivalents, when provided with one or more of the nucleotide sequences set forth in SEQ  
25 ID Nos:1, 3, 4, 5, 7, 9, 11, or 13. In particular, hybridisations may be performed using one or more nucleic acid hybridisation probes comprising at least 10 contiguous nucleotides and preferably at least 50 contiguous nucleotides derived from the nucleotide sequences set forth herein, to isolate cDNA clones, mRNA molecules, genomic clones from a genomic library (in particular genomic clones containing the entire 5' upstream region of the gene including  
30 the promoter sequence, and the entire coding region and 3'-untranslated sequences), and/or

- 11 -

synthetic oligonucleotide molecules, amongst others. The present invention clearly extends to such related sequences.

The invention further extends to any homologues, analogues or derivatives of any one or 5 more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13.

A further aspect of the present invention contemplates a nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes, a polypeptide which is required for cellulose biosynthesis in a plant, such as cellulose synthase, and which is capable 10 of hybridising under at least low stringency conditions to the nucleic acid molecule set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or to a complementary strand thereof.

As an exemplification of this embodiment, the present inventors have shown that it is possible 15 to isolate variants of the *Arabidopsis thaliana RSW1* gene sequence set forth in SEQ ID NO:3, by hybridization under low stringency conditions. Such variants include related sequences derived from *Gossypium hirsutum* (cotton), *Eucalyptus* spp. and *A. thaliana*. Additional variant are clearly encompassed by the present invention.

20 Preferably, the nucleic acid molecule further comprises a nucleotide sequence which encodes, or is complementary to a nucleotide sequence which encodes, a cellulose synthase polypeptide, more preferably a cellulose synthase which is associated with the primary or secondary plant cell wall of the plant species from which said nucleic acid molecule was derived.

25

More preferably, the nucleic acid molecule according to this aspect of the invention encodes or is complementary to a nucleic acid molecule which encodes, a polypeptide which is required for cellulose biosynthesis in a plant, such as cellulose synthase, and which is capable of hybridising under at least medium stringency conditions to the nucleic acid molecule set 30 forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or to a complementary

- 12 -

strand thereof.

Even more preferably, the nucleic acid molecule according to this aspect of the invention encodes or is complementary to a nucleic acid molecule which encodes, a polypeptide which  
5 is required for cellulose biosynthesis in a plant, such as cellulose synthase, and which is capable of hybridising under at least high stringency conditions to the nucleic acid molecule set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or to a complementary strand thereof.

- 10 For the purposes of defining the level of stringency, a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. Generally, the stringency is increased by reducing the concentration of SSC buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. A medium stringency comprises a hybridisation and/or a wash carried out in
- 15 0.2xSSC-2xSSC buffer, 0.1% (w/v) SDS at 42°C to 65°C, while a high stringency comprises a hybridisation and/or a wash carried out in 0.1xSSC-0.2xSSC buffer, 0.1% (w/v) SDS at a temperature of at least 55°C. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of further clarification only, reference to the parameters affecting hybridisation between nucleic acid molecules is found in pages
- 20 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

In an even more preferred embodiment of the invention, the isolated nucleic acid molecule further comprises a sequence of nucleotides which is at least 40% identical to at least 10 contiguous nucleotides derived from any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or  
25 13, or a complementary strand thereof.

Still more preferably, the isolated nucleic acid molecule further comprises a sequence of nucleotides which is at least 40% identical to at least 50 contiguous nucleotides derived from the sequence set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a  
30 complementary strand thereof.

- 13 -

The present invention is particularly directed to a nucleic acid molecule which is capable of functioning as a cellulose gene as hereinbefore defined, for example a cellulose synthase gene such as, but not limited to, the *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* or *Eucalyptus* spp. cellulose synthase genes, amongst 5 others. The subject invention clearly contemplates additional cellulose genes to those specifically described herein which are derived from these plant species.

The invention further contemplates other sources of cellulose genes such as but not limited to, tissues and cultured cells of plant origin. Preferred plant species according to this 10 embodiment include hemp, jute, flax and woody plants including, but not limited to *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

A genetic sequence which encodes or is complementary to a sequence which encodes a polypeptide which is involved in cellulose biosynthesis may correspond to the naturally 15 occurring sequence or may differ by one or more nucleotide substitutions, deletions and/or additions. Accordingly, the present invention extends to cellulose genes and any functional genes, mutants, derivatives, parts, fragments, homologues or analogues thereof or non-functional molecules but which are at least useful as, for example, genetic probes, or primer sequences in the enzymatic or chemical synthesis of said gene, or in the generation of 20 immunologically interactive recombinant molecules.

In a particularly preferred embodiment, the cellulose genetic sequences are employed to identify and isolate similar genes from plant cells, tissues, or organ types of the same species, or from the cells, tissues, or organs of another plant species.

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According to this embodiment, there is contemplated a method for identifying a related cellulose gene or related cellulose genetic sequence, for example a cellulose synthase or cellulose synthase-like gene, said method comprising contacting genomic DNA, or mRNA, or cDNA with a hybridisation effective amount of a first cellulose genetic sequence 30 comprising any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a complementary

- 14 -

sequence, homologue, analogue or derivative thereof derived from at least 10 contiguous nucleotides of said first sequence, and then detecting said hybridisation.

Preferably, the first genetic sequence comprises at least 50 contiguous nucleotides, even more 5 preferably at least 100 contiguous nucleotides and even more preferably at least 500 contiguous nucleotides, derived from any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a complementary strand, homologue, analogue or derivative thereof.

The related cellulose gene or related cellulose genetic sequence may be in a recombinant 10 form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell.

Preferably, the related cellulose gene or related cellulose genetic sequence is derived from a plant species, such as a monocotyledonous plant or a dicotyledonous plant selected from the list comprising *Arabidopsis thaliana*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., hemp, jute, flax, and woody plants 15 including, but not limited to *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

More preferably, related cellulose gene or related cellulose genetic sequence is derived from a plant which is useful in the fibre or timber industries, for example *Gossypium hirsutum* (cotton), hemp, jute, flax, *Eucalyptus* spp. or *Pinus* spp., amongst others. Alternatively, the 20 related cellulose gene or related cellulose genetic sequence is derived from a plant which is useful in the cereal or starch industry, for example wheat, barley, rice or maize, amongst others.

In a particularly preferred embodiment, the first cellulose genetic sequence is labelled with 25 a reporter molecule capable of giving an identifiable signal (e.g. a radioisotope such as <sup>32</sup>P or <sup>35</sup>S or a biotinylated molecule).

An alternative method contemplated in the present invention involves hybridising two nucleic acid "primer molecules" to a nucleic acid "template molecule" which comprises a related 30 cellulose gene or related cellulose genetic sequence or a functional part thereof, wherein the

- 15 -

first of said primers comprises contiguous nucleotides derived from any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13 or a homologue, analogue or derivative thereof and the second of said primers comprises contiguous nucleotides complementary to any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13. Specific nucleic acid molecule copies of the 5 template molecule are amplified enzymatically in a polymerase chain reaction, a technique that is well known to one skilled in the art.

In a preferred embodiment, each nucleic acid primer molecule is at least 10 nucleotides in length, more preferably at least 20 nucleotides in length, even more preferably at least 30 10 nucleotides in length, still more preferably at least 40 nucleotides in length and even still more preferably at least 50 nucleotides in length.

Furthermore, the nucleic acid primer molecules consists of a combination of any of the nucleotides adenine, cytidine, guanine, thymidine, or inosine, or functional analogues or 15 derivatives thereof which are at least capable of being incorporated into a polynucleotide molecule without having an inhibitory effect on the hybridisation of said primer to the template molecule in the environment in which it is used.

Furthermore, one or both of the nucleic acid primer molecules may be contained in an 20 aqueous mixture of other nucleic acid primer molecules, for example a mixture of degenerate primer sequences which vary from each other by one or more nucleotide substitutions or deletions. Alternatively, one or both of the nucleic acid primer molecules may be in a substantially pure form.

25 The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the nucleic acid

template molecule is derived from a plant cell, tissue or organ, in particular a cell, tissue or organ derived from a plant selected from the list comprising *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* and *Eucalyptus* spp., hemp, jute, flax, and woody plants including, but not limited to *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

Those skilled in the art will be aware that there are many known variations of the basic polymerase chain reaction procedure, which may be employed to isolate a related cellulose gene or related cellulose genetic sequence when provided with the nucleotide sequences set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13. Such variations are discussed, for example, in McPherson *et al* (1991). The present invention extends to the use of all such variations in the isolation of related cellulose genes or related cellulose genetic sequences using the nucleotide sequences embodied by the present invention.

- 15 The isolated nucleic acid molecule according to any of the further embodiments may be cloned into a plasmid or bacteriophage molecule, for example to facilitate the preparation of primer molecules or hybridisation probes or for the production of recombinant gene products. Methods for the production of such recombinant plasmids, cosmids, bacteriophage molecules or other recombinant molecules are well-known to those of ordinary skill in the art and can be accomplished without undue experimentation. Accordingly, the invention further extends to any recombinant plasmid, bacteriophage, cosmid or other recombinant molecule comprising the nucleotide sequence set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a complementary sequence, homologue, analogue or derivative thereof.
- 25 The nucleic acid molecule of the present invention is also useful for developing genetic constructs which express a cellulose genetic sequence, thereby providing for the increased expression of genes involved in cellulose biosynthesis in plants, selected for example from the list comprising *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* and *Eucalyptus* spp., hemp, jute, flax, and woody plants including, but not limited to *Pinus* spp., *Populus* spp., *Picea* spp., amongst others. The present invention

particularly contemplates the modification of cellulose biosynthesis in cotton, hemp, jute, flax, *Eucalyptus* ssp. and *Pinus* ssp., amongst others.

The present inventors have discovered that the genetic sequences disclosed herein are capable  
5 of being used to modify the level of non-crystalline  $\beta$ -1,4,-glucan, in addition to altering cellulose levels when expressed, particularly when expressed in plants cells. In particular, the *Arabidopsis thaliana rsw1* mutant has increased levels of non-crystalline  $\beta$ -1,4,-glucan, when grown at 31°C, compared to wild-type plants, grown under identical conditions. The expression of a genetic sequence described herein in the antisense orientation in transgenic  
10 plants grown at only 21°C is shown to reproduce many aspects of the *rsw1* mutant phenotype.

Accordingly, the present invention clearly extends to the modification of non-crystalline  $\beta$ -1,4,-glucan biosynthesis in plants, selected for example from the list comprising *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* ssp., *Gossypium hirsutum* and  
15 *Eucalyptus* ssp., hemp, jute, flax, and woody plants including, but not limited to *Pinus* ssp., *Populus* ssp., *Picea* spp., amongst others. The present invention particularly contemplates the modification of non-crystalline  $\beta$ -1,4,-glucan biosynthesis in cotton, hemp, jute, flax, *Eucalyptus* ssp. and *Pinus* ssp., amongst others.

20 The present invention further extends to the production and use of non-crystalline  $\beta$ -1,4-glucan and to the use of the glucan to modify the properties of plant cell walls or cotton fibres or wood fibres. Such modified properties are described herein (Example 13).

The inventors have discovered that the *rsw1* mutant has altered carbon partitioning compared  
25 to wild-type plants, resulting in significantly higher starch levels therein. The isolated nucleic acid molecules provided herein are further useful for altering the carbon partitioning in a cell. In particular, the present invention contemplates increased starch production in transgenic plants expressing the nucleic acid molecule of the invention in the antisense orientation or alternatively, expressing a ribozyme or co-suppression molecule comprising the nucleic acid  
30 sequence of the invention.

The invention further contemplates reduced starch and/or non-crystalline  $\beta$ -1,4-glucan product in transgenic plants expressing the nucleic acid molecule of the invention in the sense orientation such that cellulose production is increased therein.

5 Wherein it is desired to increase cellulose production in a plant cell, the coding region of a cellulose gene is placed operably behind a promoter, in the sense orientation, such that a cellulose gene product is capable of being expressed under the control of said promoter sequence. In a preferred embodiment, the cellulose genetic sequence is a cellulose synthase genomic sequence, cDNA molecule or protein-coding sequence.

10

In a particularly preferred embodiment, the cellulose genetic sequence comprises a sequence of nucleotides substantially the same as the sequence set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13 or a homologue, analogue or derivative thereof.

15 Wherein it is desirable to reduce the content of cellulose or to increase the content of non-crystalline  $\beta$ -1,4-glucan, the nucleic acid molecule of the present invention is expressed in the antisense orientation under the control of a suitable promoter. Additionally, the nucleic acid molecule of the invention is also useful for developing ribozyme molecules, or in co-suppression of a cellulose gene. The expression of an antisense, ribozyme or co-suppression  
20 molecule comprising a cellulose gene, in a cell such as a plant cell, fungal cell, insect cell, animal cell, yeast cell or bacterial cell, may also increase the solubility, digestibility or extractability of metabolites from plant tissues or alternatively, or increase the availability of carbon as a precursor for any secondary metabolite other than cellulose (e.g. starch or sucrose). By targeting the endogenous cellulose gene, expression is diminished, reduced or  
25 otherwise lowered to a level that results in reduced deposition of cellulose in the primary or secondary cell walls of the plant cell, fungal cell, insect cell, animal cell, yeast cell or bacterial cell, and more particularly, a plant cell. Additionally, or alternatively, the content of non-crystalline  $\beta$ -1,4-glucan is increased in such cells.

30 Co-suppression is the reduction in expression of an endogenous gene that occurs when one

or more copies of said gene, or one or more copies of a substantially similar gene are introduced into the cell. The present invention also extends to the use of co-suppression to inhibit the expression of a gene which encodes a cellulose gene product, such as but not limited to cellulose synthase. Preferably, the co-suppression molecule of the present 5 invention targets a plant mRNA molecule which encodes a cellulose synthase enzyme, for example a plant, fungus, or bacterial cellulose synthase mRNA, and more preferably a plant mRNA derived from *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* and *Eucalyptus* spp., hemp, jute, flax, or a woody plant such as *Pinus* spp., *Populus* spp., or *Picea* spp., amongst others.

10

In a particularly preferred embodiment, the gene which is targeted by a co-suppression molecule, comprises a sequence of nucleotides set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a complement, homologue, analogue or derivative thereof.

15 In the context of the present invention, an antisense molecule is an RNA molecule which is transcribed from the complementary strand of a nuclear gene to that which is normally transcribed to produce a "sense" mRNA molecule capable of being translated into a polypeptide component of the cellulose biosynthetic pathway. The antisense molecule is therefore complementary to the mRNA transcribed from a sense cellulose gene or a part 20 thereof. Although not limiting the mode of action of the antisense molecules of the present invention to any specific mechanism, the antisense RNA molecule possesses the capacity to form a double-stranded mRNA by base pairing with the sense mRNA, which may prevent translation of the sense mRNA and subsequent synthesis of a polypeptide gene product.

25 Preferably, the antisense molecule of the present invention targets a plant mRNA molecule which encodes a cellulose gene product, for example cellulose synthase. Preferably, the antisense molecule of the present invention targets a plant mRNA molecule which encodes a cellulose synthase enzyme, for example a plant mRNA derived from *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* and *Eucalyptus* spp., 30 hemp, jute, flax, or a woody plant such as *Pinus* spp., *Populus* spp., or *Picea* spp., amongst

- 20 -

others.

In a particularly preferred embodiment, the antisense molecule of the invention targets an mRNA molecule encoded by any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or  
5 a homologue, analogue or derivative thereof.

Ribozymes are synthetic RNA molecules which comprise a hybridising region complementary to two regions, each of at least 5 contiguous nucleotide bases in the target sense mRNA. In addition, ribozymes possess highly specific endoribonuclease activity, which autocatalytically  
10 cleaves the target sense mRNA. A complete description of the function of ribozymes is presented by Haseloff and Gerlach (1988) and contained in International Patent Application No. WO89/05852.

The present invention extends to ribozyme which target a sense mRNA encoding a cellulose  
15 gene product, thereby hybridising to said sense mRNA and cleaving it, such that it is no longer capable of being translated to synthesise a functional polypeptide product. Preferably, the ribozyme molecule of the present invention targets a plant mRNA molecule which encodes a cellulose synthase enzyme, for example a plant mRNA derived from *Arabidopsis thaliana*,  
*Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., hemp, jute, flax, or a  
20 woody plant such as *Pinus* spp., *Populus* spp., or *Picea* spp., amongst others.

In a particularly preferred embodiment, the ribozyme molecule will target an mRNA encoded by any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a homologue, analogue or derivative thereof.

25

According to this embodiment, the present invention provides a ribozyme or antisense molecule comprising at least 5 contiguous nucleotide bases derived from any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a complementary nucleotide sequence or a homologue, analogue or derivative thereof, wherein said antisense or ribozyme molecule is  
30 able to form a hydrogen-bonded complex with a sense mRNA encoding a cellulose gene

product to reduce translation thereof.

In a preferred embodiment, the antisense or ribozyme molecule comprises at least 10 to 20 contiguous nucleotides derived from any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a complementary nucleotide sequence or a homologue, analogue or derivative thereof. Although the preferred antisense and/or ribozyme molecules hybridise to at least about 10 to 20 nucleotides of the target molecule, the present invention extends to molecules capable of hybridising to at least about 50-100 nucleotide bases in length, or a molecule capable of hybridising to a full-length or substantially full-length mRNA encoded by a cellulose gene.

such as a cellulose synthase gene.

Those skilled in the art will be aware of the necessary conditions, if any, for selecting or preparing the antisense or ribozyme molecules of the invention.

It is understood in the art that certain modifications, including nucleotide substitutions amongst others, may be made to the antisense and/or ribozyme molecules of the present invention, without destroying the efficacy of said molecules in inhibiting the expression of a gene encoding a cellulose gene product such as cellulose synthase. It is therefore within the scope of the present invention to include any nucleotide sequence variants, homologues, analogues, or fragments of the said gene encoding same, the only requirement being that said nucleotide sequence variant, when transcribed, produces an antisense and/or ribozyme molecule which is capable of hybridising to a sense mRNA molecule which encodes a cellulose gene product.

Gene targeting is the replacement of an endogenous gene sequence within a cell by a related DNA sequence to which it hybridises, thereby altering the form and/or function of the endogenous gene and the subsequent phenotype of the cell. According to this embodiment, at least a part of the DNA sequence defined by any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a related cellulose genetic sequence, may be introduced into target cells containing an endogenous cellulose gene, thereby replacing said endogenous cellulose gene.

According to this embodiment, the polypeptide product of said cellulose genetic sequence possesses different catalytic activity and/or expression characteristics, producing in turn modified cellulose deposition in the target cell. In a particularly preferred embodiment of the invention, the endogenous cellulose gene of a plant is replaced with a gene which is merely capable of producing non-crystalline  $\beta$ -1,4-glucan polymers or alternatively which is capable of producing a modified cellulose having properties similar to synthetic fibres such as rayon, in which the  $\beta$ -1,4-glucan polymers are arranged in an antiparallel configuration relative to one another.

- 10 The present invention extends to genetic constructs designed to facilitate expression of a cellulose genetic sequence which is identical, or complementary to the sequence set forth in any one or more of SEQ ID Nos:1, 3, 4, 5, 7, 9, 11 or 13, or a functional derivative, part, homologue, or analogue thereof, or a genetic construct designed to facilitate expression of a sense molecule, an antisense molecule, ribozyme molecule, co-suppression molecule, or
- 15 gene targeting molecule containing said genetic sequence.

The said genetic construct of the present invention comprises the foregoing sense, antisense, or ribozyme, or co-suppression nucleic acid molecule, or gene-targeting molecule, placed operably under the control of a promoter sequence capable of regulating the expression of the

- 20 said nucleic acid molecule in a prokaryotic or eukaryotic cell, preferably a plant cell. The said genetic construct optionally comprises, in addition to a promoter and sense, or antisense, or ribozyme, or co-suppression, or gene-targeting nucleic acid molecule, a terminator sequence.

- 25 The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in plant cells are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals
- 30 and/or plants. Examples of terminators particularly suitable for use in the genetic constructs

of the present invention include the nopaline synthase (NOS) gene terminator of *Agrobacterium tumefaciens*, the terminator of the Cauliflower mosaic virus (CaMV) 35S gene, and the zein gene terminator from *Zea mays*.

- 5 Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external  
10 stimuli, or in a tissue-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5', of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.
- 15 In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of said sense, antisense, or ribozyme; or co-suppression nucleic acid molecule, in a plant cell. Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression of a sense antisense, ribozyme or co-suppression molecule and/or  
20 to alter the spatial expression and/or temporal expression of said sense or antisense, or ribozyme, or co-suppression, or gene-targeting molecule. For example, regulatory elements which confer copper inducibility may be placed adjacent to a heterologous promoter sequence driving expression of a sense, or antisense, or ribozyme, or co-suppression, or gene-targeting molecule, thereby conferring copper inducibility on the expression of said molecule.  
25 Placing a sense or ribozyme, or antisense, or co-suppression, or gene-targeting molecule under the regulatory control of a promoter sequence means positioning the said molecule such that expression is controlled by the promoter sequence. Promoters are generally positioned 5' (upstream) to the genes that they control. In the construction of heterologous  
30 promoter/structural gene combinations it is generally preferred to position the promoter at a

distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a 5 regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Examples of promoters suitable for use in genetic constructs of the present invention include 10 viral, fungal, bacterial, animal and plant derived promoters capable of functioning in prokaryotic or eukaryotic cells. Preferred promoters are those capable of regulating the expression of the subject cellulose genes of the invention in plants cells, fungal cells, insect cells, yeast cells, animal cells or bacterial cells, amongst others. Particularly preferred promoters are capable of regulating expression of the subject nucleic acid molecules in plant 15 cells. The promoter may regulate the expression of the said molecule constitutively, or differentially with respect to the tissue in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or plant pathogens, or metal ions, amongst others. Preferably, the promoter is capable of regulating expression of a sense, or ribozyme, or antisense, or co- 20 suppression molecule or gene targeting, in a plant cell. Examples of preferred promoters include the CaMV 35S promoter, NOS promoter, octopine synthase (OCS) promoter and the like.

In a most preferred embodiment, the promoter is capable of expression in any plant cell, such 25 as, but not limited to a plant selected from the list comprising *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* and *Eucalyptus* spp., hemp, jute, flax, and woody plants including, but not limited to *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

30 In a particularly preferred embodiment, the promoter may be derived from a genomic clone

- 25 -

encoding a cellulose gene product, in particular the promoter contained in the sequence set forth in SEQ ID NO:3 or SEQ ID NO:4. Preferably, the promoter sequence comprises nucleotide 1 to about 1900 of SEQ ID NO:3 or nucleotides 1 to about 700 of SEQ ID NO:4 or a homologue, analogue or derivative capable of hybridizing thereto under at least low 5 stringency conditions.

Optionally, the genetic construct of the present invention further comprises a terminator sequence.

10 In an exemplification of this embodiment, there is provided a binary genetic construct comprising the isolated nucleotide sequence of nucleotides set forth in SEQ ID NO:3. There is also provided a genetic construct comprising the isolated nucleotide sequence of nucleotides set forth in SEQ ID NO:1, in the antisense orientation, placed operably in connection with the CaMV 35S promoter.

15

In the present context, the term "in operable connection with" means that expression of the isolated nucleotide sequence is under the control of the promoter sequence with which it is connected, regardless of the relative physical distance of the sequences from each other or their relative orientation with respect to each other.

20

An alternative embodiment of the invention is directed to a genetic construct comprising a promoter or functional derivative, part, fragment, homologue, or analogue thereof, which is capable of directing the expression of a polypeptide early in the development of a plant cell at a stage when the cell wall is developing, such as during cell expansion or during cell division. In a particularly preferred embodiment, the promoter is contained in the sequence set forth in SEQ ID NO:3 or SEQ ID NO:4. Preferably, the promoter sequence comprises nucleotide 1 to about 1900 of SEQ ID NO:3 or nucleotides 1 to about 700 of SEQ ID NO:4 or a homologue, analogue or derivative capable of hybridizing thereto under at least low stringency conditions.

30

The polypeptide may be a reporter molecule which is encoded by a gene such as the bacterial β-glucuronidase gene or chloramphenicol acetyltransferase gene or alternatively, the firefly luciferase gene. Alternatively, the polypeptide may be encoded by a gene which is capable of producing a modified cellulose in the plant cell when placed in combination with the 5 normal complement of cellulose genes which are expressible therein, for example it may be a cellulose-like gene obtained from a bacterial or fungal source or a cellulose gene obtained from a plant source.

The genetic constructs of the present invention are particularly useful in the production of 10 crop plants with altered cellulose content or structure. In particular, the rate of cellulose deposition may be reduced leading to a reduction in the total cellulose content of plants by transferring one or more of the antisense, ribozyme or co-suppression molecules described *supra* into a plant or alternatively, the same or similar end-result may be achieved by replacing an endogenous cellulose gene with an inactive or modified cellulose gene using 15 gene-targeting approaches. The benefits to be derived from reducing cellulose content in plants are especially apparent in food and fodder crops such as, but not limited to maize, wheat, barley, rye, rice, barley, millet or sorghum, amongst others where improved digestibility of said crop is desired. The foregoing antisense, ribozyme or co-suppression molecules are also useful in producing plants with altered carbon partitioning such that 20 increased carbon is available for growth, rather than deposited in the form of cellulose.

Alternatively, the introduction to plants of additional copies of a cellulose gene in the 'sense' orientation and under the control of a strong promoter is useful for the production of plants with increased cellulose content or more rapid rates of cellulose biosynthesis. Accordingly, 25 such plants may exhibit a range of desired traits including, but not limited to modified strength and/or shape and/or properties of fibres, cell and plants, increased protection against chemical, physical or environmental stresses such as dehydration, heavy metals (e.g. cadmium) cold, heat or wind, increased resistance to attack by pathogens such as insects, nematodes and the like which physically penetrate the cell wall barrier during 30 invasion/infection of the plant.

Alternatively, the production of plants with altered physical properties is made possible by the introduction thereto of altered cellulose gene(s). Such plants may produce  $\beta$ -1,4-glucan which is either non-crystalline or shows altered crystallinity. Such plants may also exhibit a range of desired traits including but not limited to, altered dietary fibre content, altered 5 digestibility and degradability or producing plants with altered extractability properties.

- Furthermore, genetic constructs comprising a plant cellulose gene in the 'sense' orientation may be used to complement the existing range of cellulose genes present in a plant, thereby altering the composition or timing of deposition of cellulose deposited in the cell wall of said 10 plant. In a preferred embodiment, the cellulose gene from one plant species or a  $\beta$ -1,4-glucan synthase gene from a non-plant species is used to transform a plant of a different species, thereby introducing novel cellulose biosynthetic metabolism to the second-mentioned plant species.
- 15 In a related embodiment, a recombinant fusion polypeptide may be produced containing the active site from one cellulose gene product fused to another cellulose gene product, wherein said fusion polypeptide exhibits novel catalytic properties compared to either 'parent' polypeptide from which it is derived. Such fusion polypeptides may be produced by conventional recombinant DNA techniques known to those skilled in the art, either by 20 introducing a recombinant DNA capable of expressing the entire fusion polypeptide into said plant or alternatively, by a gene-targeting approach in which recombination at the DNA level occurs *in vivo* and the resultant gene is capable of expressing a recombinant fusion polypeptide.
- 25 The present invention extends to all transgenic methods and products described *supra*, including genetic constructs.

The recombinant DNA molecule carrying the sense, antisense, ribozyme or co-suppression molecule of the present invention and/or genetic construct comprising the same, may be 30 introduced into plant tissue, thereby producing a "transgenic plant", by various techniques

known to those skilled in the art. The technique used for a given plant species or specific type of plant tissue depends on the known successful techniques. Means for introducing recombinant DNA into plant tissue include, but are not limited to, transformation (Paszkowski *et al.*, 1984), electroporation (Fromm *et al.*, 1985), or microinjection of the 5 DNA (Crossway *et al.*, 1986), or T-DNA-mediated transfer from *Agrobacterium* to the plant tissue. Representative T-DNA vector systems are described in the following references: An *et al.* (1985); Herrera-Estrella *et al.* (1983a,b); Herrera-Estrella *et al.* (1985). Once introduced into the plant tissue, the expression of the introduced gene may be assayed in a transient expression system, or it may be determined after selection for stable integration 10 within the plant genome. Techniques are known for the *in vitro* culture of plant tissue, and in a number of cases, for regeneration into whole plants. Procedures for transferring the introduced gene from the originally transformed plant into commercially useful cultivars are known to those skilled in the art.

15 A still further aspect of the present invention extends to a transgenic plant such as a crop plant, carrying the foregoing sense, antisense, ribozyme, co-suppression, or gene-targeting molecule and/or genetic constructs comprising the same. Preferably, the transgenic plant is one or more of the following: *Arabidopsis thaliana*, *Oryza sativa*, wheat, barley, maize, *Brassica* spp., *Gossypium hirsutum* and *Eucalyptus* spp., hemp, jute, flax, *Pinus* spp., 20 *Populus* spp., or *Picea* spp. Additional species are not excluded.

The present invention further extends to the progeny of said transgenic plant.

Yet another aspect of the present invention provides for the expression of the subject genetic 25 sequence in a suitable host (e.g. a prokaryote or eukaryote) to produce full length or non-full length recombinant cellulose gene products.

Hereinafter the term "cellulose gene product" shall be taken to refer to a recombinant product of a cellulose gene as hereinbefore defined. Accordingly, the term "cellulose gene product" 30 includes a polypeptide product of any gene involved in the cellulose biosynthetic pathway in

plants, such as, but not limited to a cellulose synthase gene product.

Preferably, the recombinant cellulose gene product comprises an amino acid sequence having the catalytic activity of a cellulose synthase polypeptide or a functional mutant, derivative 5 part, fragment, or analogue thereof.

In a particularly preferred embodiment of the invention, the recombinant cellulose gene product comprises a sequence or amino acids that is at least 40% identical to any one or more of SEQ ID Nos:2, 6, 8, 10, 12 or 14, or a homologue, analogue or derivative thereof.

10

Single and three-letter abbreviations used for amino acid residues contained in the specification are provided in Table 1.

In the present context, "homologues" of an amino acid sequence refer to those polypeptides, 15 enzymes or proteins which have a similar catalytic activity to the amino acid sequences described herein, notwithstanding any amino acid substitutions, additions or deletions thereto. A homologue may be isolated or derived from the same or another plant species as the species from which the polypeptides of the invention are derived.

20 "Analogues" encompass polypeptides of the invention notwithstanding the occurrence of any non-naturally occurring amino acid analogues therein.

"Derivatives" include modified peptides in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides 25 or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject peptides are particularly contemplated by the present invention. Additionally, derivatives of an amino acid sequence described herein which comprises fragments or parts of the subject amino acid sequences are within the scope of the invention, as are homopolymers or heteropolymers comprising two or more 30 copies of the subject polypeptides. Procedures for derivatizing peptides are well-known in the

- 30 -

art.

**TABLE 1**

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
5	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Cysteine	Cys	C
10	D-alanine	Dal	X
	Glutamine	Gln	Q
	Glutamic acid	Glu	E
	Glycine	Gly	G
	Histidine	His	H
15	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
20	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
25	Valine	Val	V
	Any amino acid	Xaa	X

Substitutions encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which an amino acid residue contained in a cellulose gene product is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a cellulose gene product described herein is substituted with an amino acid with different properties, such as a naturally-occurring amino acid from a different group (eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Non-conventional amino acids encompassed by the invention include, but are not limited to those listed in Table 2.

Amino acid substitutions are typically of single residues, but may be of multiple residues either clustered or dispersed.

Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to the N-terminus, the C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions and of the order of 1-4 amino acid residues.

25

A homologue, analogue or derivative of a cellulose gene product as referred to herein may readily be made using peptide synthetic techniques well-known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Techniques for making substituent mutations at pre-determined sites using recombinant DNA technology, for example by M13 mutagenesis, are also well-known. The manipulation of nucleic acid

molecules to produce variant peptides, polypeptides or proteins which manifest as substitutions, insertions or deletions are well-known in the art.

The cellulose gene products described herein may be derivatized further by the inclusion or  
5 attachment thereto of a protective group which prevents, inhibits or slows proteolytic or cellular degradative processes. Such derivatization may be useful where the half-life of the subject polypeptide is required to be extended, for ample to increase the amount of cellulose produced in a primary or secondary cell wall of a plant cell or alternatively, to increase the amount of protein produced in a bacterial or eukaryotic expression system. Examples of  
10 chemical groups suitable for this purpose include, but are not limited to, any of the non-conventional amino acid residues listed in Table 2, in particular a D-stereoisomer or a methylated form of a naturally-occurring amino acid listed in Table 1. Additional chemical groups which are useful for this purpose are selected from the list comprising aryl or heterocyclic N-acyl substituents, polyalkylene oxide moieties, desulphatohirudin mureins,  
15 alpha-mureins, alpha-aminophosphonic acids, water-soluble polymer groups such as polyethylene glycol attached to sugar residues using hydrazone or oxime groups, benzodiazepine dione derivatives, glycosyl groups such as beta-glycosylamine or a derivative thereof, isocyanate conjugated to a polyol functional group or polyoxyethylene polyol capped with diisocyanate, amongst others. Similarly, a cellulose gene product or a homologue,  
20 analogue or derivative thereof may be cross-linked or fused to itself or to a protease inhibitor peptide, to reduce susceptibility of said molecule to proteolysis.

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5 $\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methyleasparagine	Nmasn
10 aminoisobutyric acid	Aib	L-N-methyleaspartic acid	Nmasp
aminonorbornyl- carboxylate	Norb	L-N-methylcysteine	Nmcys
cyclohexylalanine	Chexa	L-N-methylglutamine	Nmgln
cyclopentylalanine	Cpen	L-N-methylglutamic acid	Nmglu
15 D-alanine	Dal	L-N-methylhistidine	Nmhis
D-arginine	Darg	L-N-methyleucine	Nmleu
D-aspartic acid	Dasp	L-N-methylethionine	Nmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20 D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
25 D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30 D-threonine	Dthr	L-norleucine	Nle

D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgabu
D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
5 D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
D- $\alpha$ -methylasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
D- $\alpha$ -methylaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
10 D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
D- $\alpha$ -methylleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
15 D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Ncbut
D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
20 D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D- $\alpha$ -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D- $\alpha$ -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
25 D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
30 D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser

D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
5 D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nieu	D-N-methylthreonine	Dnmthr
10 D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-naphthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
$\gamma$ -aminobutyric acid	Gabu	N-( <i>p</i> -hydroxyphenyl)glycine	Nhtyr
L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
15 L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methylasparagine	Masn
L- $\alpha$ -methylaspartate	Masp	L- $\alpha$ -methyl- <i>t</i> -butylglycine	Mtbug
L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
20 L- $\alpha$ -methylglutamine	Mglu	L- $\alpha$ -methylglutamate	Mglu
L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- $\alpha$ -methylleucine	Mleu	L- $\alpha$ -methyllysine	Mlys
L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
25 L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr
L- $\alpha$ -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe

- 36 -

N-(N-(2,2-diphenylethyl)

Nnbhm

carbamylmethyl)glycine

N-(N-(3,3-diphenylpropyl)

Nnbhe

carbamylmethyl)glycine

1-carboxy-1-(2,2-diphenyl-

Nmbc

ethylamino)cyclopropane

5

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In an alternative embodiment of the invention, the recombinant cellulose gene product is characterised by at least one functional  $\beta$ -glycosyl transferase domain contained therein.

10

The term " $\beta$ -glycosyl transferase domain" as used herein refers to a sequence of amino acids which is highly conserved in different processive enzymes belonging to the class of glycosyl transferase enzymes (Saxena *et al.*, 1995), for example the bacterial  $\beta$ -1,4-glycosyl transferase enzymes and plant cellulose synthase enzymes amongst others, wherein said 15 domain possesses a putative function in contributing to or maintaining the overall catalytic activity, substrate specificity or substrate binding of an enzyme in said enzyme class. The  $\beta$ -glycosyl transferase domain is recognisable by the occurrence of certain amino acid residues at particular locations in a polypeptide sequence, however there is no stretch of contiguous amino acid residues comprised therein.

20

As a consequence of the lack of contiguity in a  $\beta$ -glycosyl transferase domain, it is not a straightforward matter to isolate a cellulose gene by taking advantage of the presence of a  $\beta$ -glycosyl transferase domain in the polypeptide encoded by said gene. For example, the  $\beta$ -glycosyl transferase domain would not be easily utilisable as a probe to facilitate the rapid 25 isolation of all  $\beta$ -glycosyl transferase genetic sequences from a particular organism and then to isolate from those genetic sequences a cellulose gene such as cellulose synthase.

In a preferred embodiment, the present invention provides an isolated polypeptide which:

(i)contains at least one structural  $\beta$ -glycosyl transferase domain as hereinbefore

30 defined; and

- 37 -

(ii) has at least 40% amino acid sequence similarity to at least 20 contiguous amino acid residues set forth in any one or more of SEQ ID Nos:2, 6, 8, 10, 12 or 14, or a homologue, analogue or derivative thereof.

5 More preferably, the polypeptide of the invention is at least 40% identical to at least 50 contiguous amino acid residues, even more preferably at least 100 amino acid residues of any one or more of SEQ ID Nos:2, 6, 8, 10, 12 or 14, or a homologue, analogue or derivative thereof.

10 In a particularly preferred embodiment, the percentage similarity to any one or more of SEQ ID Nos:2, 6, 8, 10, 12 or 14 is at least 50-60%, more preferably at least 65-70%, even more preferably at least 75-80% and even more preferably at least 85-90%, including about 91% or 95%.

15 In a related embodiment, the present invention provides a "sequencably pure" form of the amino acid sequence described herein. "Sequencably pure" is hereinbefore described as substantially homogeneous to facilitate amino acid determination.

In a further related embodiment, the present invention provides a "substantially homogeneous" form of the subject amino acid sequence, wherein the term "substantially homogeneous" is hereinbefore defined as being in a form suitable for interaction with an immunologically interactive molecule. Preferably, the polypeptide is at least 20% homogeneous, more preferably at least 50% homogeneous, still more preferably at least 75% homogeneous and yet still more preferably at least about 95-100% homogenous, in terms of activity per microgram of total protein in the protein preparation.

The present invention further extends to a synthetic peptide of at least 5 amino acid residues in length derived from or comprising a part of the amino acid sequence set forth in any one or more of SEQ ID Nos:2, 6, 8, 10, 12 or 14, or having at least 40% similarity thereto.

Those skilled in the art will be aware that such synthetic peptides may be useful in the production of immunologically interactive molecules for the preparation of antibodies or as the peptide component of an immunoassay.

5 The invention further extends to an antibody molecule such as a polyclonal or monoclonal antibody or an immunologically interactive part or fragment thereof which is capable of binding to a cellulose gene product according to any of the foregoing embodiments.

The term "antibody" as used herein, is intended to include fragments thereof which are also 10 specifically reactive with a polypeptide of the invention. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as for whole antibodies. For example, F(ab')<sub>2</sub> fragments can be generated by treating antibody with pepsin. The resulting F(ab')<sub>2</sub> fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

15

Those skilled in the art will be aware of how to produce antibody molecules when provided with the cellulose gene product of the present invention. For example, by using a polypeptide of the present invention polyclonal antisera or monoclonal antibodies can be made using standard methods. A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an 20 immunogenic form of the polypeptide which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a polypeptide include conjugation to carriers or other techniques well known in the art. For example, the polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used with 25 the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired IgG molecules corresponding to the polyclonal antibodies may be isolated from the sera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested 30 from an immunized animal and fused with myeloma cells by standard somatic cell fusion

- 39 -

procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein (1975) as well as other techniques such as the human B-cell hybridoma technique (Kozbor *et al.*, 1983), the EBV-hybridoma technique to produce human monoclonal antibodies 5 (Cole *et al.*, 1985), and screening of combinatorial antibody libraries (Huse *et al.*, 1989). Hybridoma cells can be screened immunochemically for production of antibodies which are specifically reactive with the polypeptide and monoclonal antibodies isolated.

As with all immunogenic compositions for eliciting antibodies, the immunogenically effective 10 amounts of the polypeptides of the invention must be determined empirically. Factors to be considered include the immunogenicity of the native polypeptide, whether or not the polypeptide will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier and route of administration for the composition, i.e. intravenous, intramuscular, subcutaneous, *etc.*, and the number of immunizing doses to be administered. Such factors are 15 known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue experimentation.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed 20 above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody.

The present invention is further described by reference to the following non-limiting Figures and Examples.

- 40 -

In the Figures:

Figure 1 is a photographic representation showing the inflorescence length of wild-type *Arabidopsis thaliana* Columbia plants (plants 1 and 3) and *rsw1* plants (plants 2 and 4) 5 grown at 21°C (plants 1 and 2) or 31°C. Plants were grown initially at 21°C until bolting commenced, the bolts were removed and the re-growth followed in plants grown at each temperature.

Figure 2 is a photographic representation of a cryo-scanning electron micrograph showing 10 misshapen epidermal cells in the cotyledons and hypocotyl of the *rsw1* mutant when grown at 31°C for 10 days.

Figure 3 is a graphical representation of a gas chromatograph of alditol acetates of methylated sugars from a cellulose standard (top panel) and from the neutral glucan derived 15 from shoots of *rsw1* plants grown at 31°C (lower panel). The co-incident peaks show that the *rsw1* glucan is 1,4-linked.

Figure 4 is a schematic representation of the contiguous region of *Arabidopsis thaliana* chromosome 4 (stippled box) between the cosmid markers g8300 and 06455, showing the 20 location of overlapping YAC clones (open boxes) within the contiguous region. The position of the *RSW1* locus is also indicated, approximately 1.2cM from g8300 and 0.9cM from 06455. The scale indicates 100kb in length. L, left-end of YAC; R, right-end of YAC. Above the representation of chromosome 4, the YAC fragments and cosmid clone fragments used to construct the contiguous region are indicated, using a prefix designation 25 corresponding to the YAC or cosmid from which the fragments were obtained( eg yUP9E3, yUP20B12, etc) and a suffix designation indicating whether the fragment corresponds to the right-end (RE) or left-end (LE) of the YAC clone; N, North; S, South; CAPS, cleaved amplified polymorphic sequence (Konieczny and Ausubel, 1993) version of the g8300 marker.

- 41 -

Figure 5 is a schematic representation of a restriction map of construct 23H12 between the left T-DNA border (LB) and right T-DNA border (RB) sequences (top solid line), showing the position of the *Arabidopsis thaliana RSW1* locus (stippled box). The line at the top of the figure indicates the region of 23H12 which is contained in construct pRSW1. The 5 structure of the *RSW1* gene between the translation start (ATG) and translation stop (TAG) codons is indicated at the bottom of the figure. Exons are indicated by filled boxes; introns are indicated by the solid black line. The alignment of EST clone T20782 to the 3'-end of the *RSW1* gene, from near the end of exon 7 to the end of exon 14, is also indicated at the bottom of the figure. Restriction sites within 23H12 are as follows: B, *Bam*HI; E, *Eco*RI; 10 H, *Hind*III; S, *Sal*I; Sm, *Sma*I.

Figure 6 is a photographic representation showing complementation of the radial root swelling phenotype of the *rsw1* mutant by transformation with construct 23H12. The *rsw1* mutant was transformed with 23H12 as described in Example 6. Transformed *rsw1* plants 15 (centre group of three seedlings), untransformed *rsw1* plants (left group of three seedlings) and untransformed *A.thaliana* Columbia plants (right group of three seedlings) were grown at 21°C for 5 days and then transferred to 31°C for a further 2 days, after which time the degree of root elongation and radial root swelling was determined.

20 Figure 7 is a photographic representation comparing wild-type *Arabidopsis thaliana* Columbia plants (right-hand side of the ruler) and *A.thaliana* Columbia plants transformed with the antisense *RSW1* construct (i.e. EST T20782 expressed in the antisense orientation under control of the CaMV 35S promoter sequence; left-hand side of the ruler), showing inflorescence shortening at 21°C in plants transformed with the antisense *RSW1* construct 25 compared to untransformed Columbia plants. The phenotype of the antisense plants at 21°C is similar to the phenotype of the *rsw1* mutant at 31°C. Inflorescence height is indicated in millimetres.

Figure 8 is a schematic representation showing the first 90 amino acid residues of 30 *Arabidopsis thaliana RSW1* aligned to the amino acid sequences of homologous polypeptides

- 42 -

from *A. thaliana* and other plant species. The shaded region indicates highly conserved sequences. Ath-A and Ath-B are closely related *Arabidopsis thaliana* cDNA clones identified by hybridisation screening using part of the RSW1 cDNA as a probe. S0542, rice EST clone (MAFF DNA bank, Japan); celA1 and celA2, cotton cDNA sequences expressed in cotton fibre (Pear *et al.*, 1996); SOYSTF1A and SOYSTF1B, putative soybean bZIP transcription factors. Amino acid designations are as indicated in Table 1 incorporated herein. Conserved cysteine residues are indicated by the asterisk.

Figure 9 is a schematic representation showing the alignment of the complete amino acid sequence of *Arabidopsis thaliana* RSW1 to the amino acid sequences of homologous polypeptides from *A. thaliana* and other plant species. The shaded region indicates highly conserved sequences. Ath-A and Ath-B are closely related *Arabidopsis thaliana* cDNA clones identified by hybridisation screening using part of the RSW1 cDNA as a probe. S0542, rice EST clone (MAFF DNA bank, Japan); celA1, cotton genetic sequence (Pear *et al.*, 1996); D48636, a partial cDNA clone obtained from rice (Pear *et al.*, 1996). Amino acid designations are as indicated in Table 1 incorporated herein. Numbering indicates the amino acid position in the RSW1 sequence.

Figure 10 is a schematic representation of the RSW1 polypeptide, showing the positions of putative transmembrane helices (hatched boxes), cysteine-rich region (Cys) and aspartate residues (D) and the QVLRW signature which are conserved between RSW1 and related amino acid sequences. Regions of RSW1 which are highly-conserved between putative cellulose biosynthesis polypeptides are indicated by the dark-shaded boxes, while less-conserved regions are indicated by the light-shaded boxes.

25

Figure 11 is a photographic representation of a Southern blot hybridisation of the 5'- end of the *Arabidopsis thaliana* RSW1 cDNA to *Bgl*II-digested DNA derived from *A. thaliana* (lane 1) and cotton (lane 2). Hybridisations were carried out under low stringency conditions at 55°C. Arrows indicate the positions of hybridising bands.

30

**EXAMPLE 1**

**CHARACTERISATION OF THE CELLULOSE-DEFICIENT**

***Arabidopsis thaliana* MUTANT *rsw1***

**5 1. Morphology**

The *Arabidopsis thaliana rsw1* mutant was produced in a genetic background comprising the ecotype Columbia.

The altered root cell-shape and temperature sensitivity of the root morphology of the 10 *Arabidopsis thaliana* mutant *rsw1* are disclosed, among other morphological mutants, by Baskin *et al.* (1992).

As shown in Figure 1, the present inventors have shown that the *rsw1* mutant exhibits the surprising phenotype of having reduced inflorescence height when grown at 31°C, compared 15 to wild-type Columbia plants grown under similar conditions. In contrast, when grown at 21°C, the inflorescence height of *rsw1* is not significantly different from wild type plants grown under similar conditions, indicating that the shoot phenotype of *rsw1* is conditional and temperature-dependent.

20 Furthermore, cryo-scanning electron microscopy of the epidermal cells of the *rsw1* mutant indicates significant abnormality in cell shape, particularly in respect of those epidermal cells forming the leaves, hypocotyl and cotyledons, when the seedlings are grown at 31°C (Figure 2).

25 Rosettes (terminal complexes) are the putative hexameric cellulose synthase complexes of higher plant plasma membranes (Herth, 1985). Freeze-fractured root cells of *Arabidopsis thaliana rsw1* plants grown at 18°C show cellulose microfibrils and rosettes on the PF face of the plasma membrane that resembles those of wild-type *A. thaliana* and other angiosperms. Transferring the *rsw1* mutant to 31°C reduces the number of rosettes in the 30 mutant within 30 min, leading to extensive loss after 3 hours. Plasma membrane particles

align in rows on prolonged exposure to the restrictive temperature. In contrast, there is no change in the appearance of cortical microtubules that align cellulose microfibrils, or of Golgi bodies that synthesise other wall polysaccharides and assemble rosettes.

### 5 2. Carbohydrate content

The effect of mutations in the *RSW1* gene on the synthesis of cellulose and other carbohydrates was assessed by measuring *in vivo* incorporation of  $^{14}\text{C}$  (supplied as uniformly labelled glucose) into various cell wall fractions. Wild type (*RSW1*) and homozygous mutant *rsw1* seed were germinated at 21°C on agar containing Hoagland's nutrients and 1% (w/v) unlabelled glucose. After 5 d, half of the seedlings were transferred to 31°C for 1 d while the remainder were maintained at 21°C for the same time. Seedlings were covered with a solution containing Hoagland's nutrients and  $^{14}\text{C}$ -glucose and incubated for a further 3 h at the same temperature. Rinsed roots and shoots were separated and frozen in liquid nitrogen. Tissue was homogenised in cold, 0.5 M potassium phosphate buffer (0.5M  $\text{KH}_2\text{PO}_4$ , pH7.0) and a crude cell wall fraction collected by centrifugation at 2800 rpm. The wall fraction was extracted with chloroform/methanol [1:1 (v/v)] at 40°C for 1 hour, followed by a brief incubation at 150°C, to remove lipids. The pellet was washed successively with 2ml methanol, 2ml acetone and twice with 2ml of deionised water. Finally, the pellet was extracted successively with dimethyl sulphoxide under nitrogen to remove starch; 0.5% ammonium oxalate to remove pectins; 0.1 M KOH and 3 mg/ml  $\text{NaBH}_4$  and then with 4 M KOH and 3 mg/ml  $\text{NaBH}_4$  to extract hemicelluloses; boiling acetic acid/nitric acid/water [8:1:2 (v/v)], to extract any residual non-cellulosic carbohydrates and leave crystalline cellulose as the final insoluble pellet (Updegraph, 1969). All fractions were analysed by liquid scintillation counting and the counts in each fraction from the mutant were expressed as a percentage of the counts in the wild type under the same conditions.

As shown in Table 3, mutant and wild type plants behave in quite similar fashion at 21°C (the permissive temperature) whereas, at the restrictive temperature of 31°C, the incorporation of  $^{14}\text{C}$  into cellulose is severely inhibited (to 36% of wild type) by the *rsw1* mutation. The data in Table 3 indicate that cellulose synthesis is specifically inhibited in

- 45 -

the *rsw1* mutant. The wild type *RSW1* gene is therefore involved quite directly in cellulose synthesis and changing its sequence by mutation changes the rate of synthesis.

5

TABLE 3

		Counts in fractions from <i>rsw1</i> plants expressed as a % of counts in comparable fraction from wild type plants					
		Pectins		Hemicelluloses		Cellulose	
		21°C	31°C	21°C	31°C	21°C	31°C
10		125	104	111	101	80	36

In homozygous mutant *rsw1* plants, the pectin fraction extracted by ammonium oxalate contained abundant glucose, atypical of true uronic acid-rich pectins. The great majority of the glucose remained in the supernatant when cetyltrimethylammonium bromide precipitated 15 the negatively charged pectins.

### 3. Non-crystalline $\beta$ -1,4-glucan content

The quantity of cellulose and the quantity of a non-crystalline  $\beta$ -1,4-glucan recovered from the ammonium oxalate fraction were determined for seedlings of wild type Columbia and for 20 backcrossed, homozygous *rsw1* that were grown for either 7 days at 21°C or alternatively, for 2 days at 21°C and 5 days at 31°C, on vertical agar plates containing growth medium (Baskin *et al.*, 1992) plus 1% (w/v) glucose, and under continuous light (90  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Roots and shoots were separated from about 150 seedlings, freeze-dried to constant weight and ground in a mortar and pestle with 3 ml of cold 0.5 M potassium phosphate buffer (pH 7.0). The 25 combined homogenate after two buffer rinses (2ml each) was centrifuged at 2800 x g for 10 min. After washing the pellet fraction twice with 2 ml buffer and twice with 2 ml distilled water, the pellet, comprising the crude cell wall fraction, and the pooled supernatants, comprising the phosphate buffer fraction were retained. The crude cell wall pellet fraction was stirred with two 3 ml aliquots of chloroform/methanol [1:1 (v/v)] for 1 hour at 40°C, 2 ml of 30 methanol at 40°C for 30 min, 2 ml of acetone for 30 min, and twice with water. The whole

procedure repeated in the case of shoots. Combined supernatants were dried in a nitrogen stream. The pellet was successively extracted with: (i) 3 ml of DMSO- water 9:1 [v/v], sealed under nitrogen, overnight with shaking, followed by two 2ml extractions using DMSO/water and three 2ml water washes; (ii) 3ml of ammonium oxalate (0.5 %) at 100°C for 1 hour, 5 followed by two water washes; (iii) 3ml of 0.1 M KOH containing 1mg/ ml sodium borohydride, for 1 hour at 25°C (repeated once for root material or twice for shoot material), with a final wash with 2 ml water; (iv) 3 ml of 4 M KOH containing 1 mg/ml sodium borohydride, for 1 hour at 25°C (repeated once for root material or twice for shoot material). The final pellet was boiled with intermittent stirring in 3 ml of acetic acid-nitric acid-water 10 [8:1:2 (v/v)] (Updegraph 1969), combined with 2 water washes, and diluted with 5 ml water.

The insoluble residue of cellulose was solubilised in 67% (v/v) H<sub>2</sub>SO<sub>4</sub>, shown to contain greater than 97% (w/v) glucose using GC/MS (Fisons AS800/MD800) of alditol acetates (Doares *et al.*, 1991) and quantified in three independent samples by anthrone/H<sub>2</sub>SO<sub>4</sub> reaction.

15 Results of GC/MS for pooled replica samples are presented in Table 4.

The non-crystalline β-1,4-glucan was recovered as the supernatant from the ammonium oxalate fraction when anionic pectins were precipitated by overnight incubation at 37°C with 2% (w/v) cetyltrimethylammonium bromide (CTAB) and collected by centrifugation at 2800 x g for 10 20 min. The glucan (250 µg/ml) or starch (Sigma; 200 µg/ml) were digested with mixtures of endocellulase (EC 3.2.1.4; Megazyme, Australia) from *Trichoderma* and almond β-glucosidase (EC 3.2.1.21; Sigma), or *Bacillus sp.* α-amylase (EC 3.2.1.1; Sigma) and rice α-glucosidase (EC 3.2.1.20; Sigma).

25 The material recovered in the supernatant from the ammonium oxalate fraction was shown to contain a pure β-1,4-glucan by demonstrating that: (i) only glucose was detectable when it was hydrolysed by 2 M TFA in a sealed tube for 1 h at 120°C in an autoclave, the supernatant (2000 g for 5 min) was dried under vacuum at 45°C to remove TFA and glucose was determined by GC/MS;

(ii) methylation (Needs and Selvendran 1993) gave a dominant peak resolved by thin layer chromatography and by GC/MS

- 47 -

that was identical to that from a cellulose standard and so indicative of 1,4-linked glucan (Figure 3); and

(iii) the endo-cellulase and  $\beta$ -1,4-glucosidase mixture released 83 % of the TFA-releasable glucose from the glucan produced by *rsw1* at 31°C while 5 the  $\alpha$ -amylase/ $\alpha$ -glucosidase mixture released no glucose from the glucan. Conversely, the  $\alpha$ -amylase/  $\alpha$ -glucosidase mixture released 95% of the TFA-releasable glucose from a starch sample, while the endo-cellulase/ $\beta$ -1,4-glucosidase mixture released no glucose from starch.

Extractability of the glucan using ammonium oxalate, and the susceptibility of the glucan to 10 endocellulase/ $\beta$ -glucosidase and TFA hydrolysis indicate that the glucan in the *rsw1* mutant is not crystalline, because it is the crystallinity of glucan which makes cellulose resistant to extraction and degradation.

Table 4 shows the quantity of glucose in cellulose determined by the anthrone/H<sub>2</sub>SO<sub>4</sub> reaction 15 and the quantity in the non-crystalline glucan after TFA hydrolysis, for shoots of wild type and mutant *rsw1* *Arabidopsis* plants. The data indicate that the production of cellulose and of the non-crystalline  $\beta$ -1,4-glucan can be manipulated by mutational changes in the *RSW1* gene.

TABLE 4

20 Glucose contents of cellulose and of the ammonium oxalate-extractable glucan

	wild type		<i>rsw1</i>	
	21°C	31°C	21°C	31°C
Cellulose	273+28	363+18*	218+20	159+19*
Glucan	22	58	24	195

All values nmol glucose mg<sup>-1</sup> plant dry weight + sd (n=3).

25 \* Differences significant at 0.001 % level.

#### 4. Starch content

The quantity of starch recovered in the DMSO fraction from roots in the experiment described above was also determined by the anthrone/H<sub>2</sub>SO<sub>4</sub> extraction (Table 5).

- 48 -

As shown in Table 5, the level of starch deposited in the *rsw1* mutant is 4-fold that detectable in the roots of wild-type plants at the restrictive temperature of 31°C. A similar rise in starch is also seen if the data are expressed as nmol glucose per plant. There is no detectable difference in deposition at starch between *rsw1* plants and wild-type plants at 5 21°C.

**TABLE 5**  
Quantity of starch (nmol glucose per mg dry weight of seedling) extracted  
from roots of *rsw1* and wild type seedlings

Temperature	Phenotype	
	Wild-type	<i>rsw1</i> mutant
21°C	22	18
31°C	37	126

The composition of cell walls in the *rsw1* mutant plant compared to wild type plants at the 15 restrictive temperature of 31°C, is summarised in Table 6.

**TABLE 6**  
Mol% composition of cell walls from shoots of *rsw1* and wild-type  
seedlings grown at 31°C

Cell wall component	Phenotype	
	Wild-type	<i>rsw1</i> mutant
Crystalline cellulose	38.4	16.5
Non-crystalline β-1,4-glucan	8.5	27.1
Pectin	37.1	36.3
Alkali-soluble	15.6	19.8
Acid-soluble	0.3	0.4

- 49 -

In conclusion, the *rsw1* mutation disassembles cellulose synthase complexes in the plasma membrane, reduces cellulose accumulation and causes  $\beta$ -1,4-glucan to accumulate in a non-crystalline form.

5

## EXAMPLE 2

### MAPPING OF YAC CLONES TO THE *rsw1* LOCUS

The *rsw1* locus in the mutant *Arabidopsis thaliana* plant described in Example 1 above was 10 mapped to chromosome 4 of *A. thaliana* using RFLP gene mapping techniques(Chang *et al.*, 1988; Nam *et al.*, 1989) to analyse the  $F_2$  or  $F_3$  progeny derived from a Columbia (Co)/Landsberg (Ler) cross. In particular, the *rsw1* mutation was shown to be linked genetically to the *ga5* locus, which is a chromosome 4 visual marker in *A. thaliana*.

15 Based on an analysis of map distances and chromosomal break points in 293  $F_2$  or  $F_3$  progeny derived from a Columbia (Co)/Landsberg (Ler) cross, *rsw1* was localised to an approximately 2.1 cM region between the RFLP markers g8300 and 06455, approximately 1.2cM south of the CAPS (cleaved amplified polymorphic sequence; Konieczny and Ausubel, 1993) version of the g8300 marker (Figure 4).

20

The interval between g8300 and 06455 in which *rsw1* residues was found to be spanned by an overlapping set of Yeast Artificial Chromosome (YAC) clones. The clones were obtained from Plant Industry, Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia. The YACs were positioned in the g8300/06455 interval by 25 hybridisation using known DNA molecular markers (from within the interval) and DNA fragments from the ends of the YACs. The length of the interval was estimated to comprise 900kb of DNA.

Refined gene mapping of recombinants within the region spanned by YAC clones established 30 the genetic distance between the RFLP marker g8300 and the *rsw1* locus.

- 50 -

The combination of genetic map distance data and the mapping of YAC clones within the region further localised the *rsw1* locus to the YAC clone designated yUPSC8.

5

### EXAMPLE 3

#### MAPPING OF cDNA CLONES TO THE YAC CLONE YUPSC8

An *Arabidopsis thaliana* cDNA clone designated T20782 was obtained from the public *Arabidopsis* Resource Centre, Ohio State University, 1735 Neil Avenue, Columbus, OH 10 43210, United States of America. The T20782 cDNA clone was localised broadly to the DNA interval on *Arabidopsis* chromosome 4 between the two markers g8300 and 06455 shown in Figure 4. Using a polymerase chain reaction (PCR) based approach DNA primers (5'-AGAACAGCAGATAACCGGA-3' and 5'-CTGAAGAAGGCTGGACAAT-3') designed to the T20782 cDNA nucleotide sequence were used to screen *Arabidopsis* YAC clone 15 libraries. The T20782 cDNA clone was found to localise to YACs (CIC1F9, CIC10E9, CIC11D9) identified on the *Arabidopsis* chromosome 4 g8300 and 06455 interval (Figure 4). The same approach was used to further localise clone T20782 to YAC clone ; UP5C8, the same YAC designated to contain the *rsw1* locus in the same chromosome interval (Figure 4).

20

Furthermore, amplification of the YAC clone yUP5C8 using primers derived from T20782 produces a 500bp fragment containing two putative exons identical to part of the T20782 nucleotide sequence, in addition to two intron sequences.

25 The cDNA T20782 was considered as a candidate gene involved in cellulose biosynthesis.

- 51 -

#### EXAMPLE 4

#### NUCLEOTIDE SEQUENCE ANALYSIS OF THE cDNA CLONE T20782

5 The nucleotide sequence of the cDNA clone T20782 is presented in SEQ ID NO: 1. The nucleotide sequence was obtained using a Dye Terminator Cycle Sequencing kit (Perkin Elmer cat. #401384) as recommended by the manufacturer. Four template clones were used for nucleotide sequencing to generate the sequence listed. The first template was the cDNA clone T20782. This template was sequenced using the following sequencing primers:

10

- a) 5'-CAATGCATTCATAGCTCCAGCCT-3'
- b) 5'-AAAAGGCTGGAGCTATGAATGCAT-3'
- c) 5'-TCACCGACAGATTCATCATAACCCG-3'
- d) 5'- GACATGGAATCACCTTAAC TGCC-3'
- 15 e) 5'-CCATTCA GTCTTGCTTCGTAACC-3'
- f) 5'-GGTTACGAAGACAAGACTGAAATGG-3'
- g) 5'-GAACCTCATAGGCATTGTGGGCTGG-3'
- h) 5'-GCAGGCTCTATATGGGTATGATCC-3'
- i) Standard M13 forward sequencing primer.
- 20 j) Standard T7 sequencing primer.

The second template clone (T20782 *Sph*I deletion clone) was constructed by creating a DNA deletion within the T20782 clone. The T20782 clone was digested with the restriction enzyme *Sph*I, the enzyme was heat-killed, the DNA ligated and electroporated into NM522 25 *E.coli* host cells. The T20782 *Sph*I deletion clone was then sequenced using a standard M13 forward sequencing primer. Two other deletion clones were made for DNA sequencing in a similar fashion but the restriction enzymes *Eco*RI and *Sma*I were used. The T20782 *Eco*RI deletion clone and the T20782 *Sma*I deletion clone were sequenced using a standard T7 sequencing primer. The DNA sequence shown in SEQ ID NO:1 is for one DNA strand 30 only however those skilled in the art will be able to generate the nucleotide sequence of the

- 52 -

complementary strand from the data provided.

The amino acid sequence encoded by clone T20782 was derived and is set forth in SEQ ID NO:2.

5

The T20782 clone encodes all but the first Aspartate (D) residue of the D, D, D, QXXRW signature conserved in the general architecture of  $\beta$ -glycosyl transferases. In particular, T20782 encodes 5 amino acid residues of the D, D, D, QXXRW signature, between amino acid positions 109 and 370 of SEQ ID NO:2. The conserved Aspartate, Aspartate, 10 Glutamine, Arginine and Tryptophan amino acid residues are shown below, in bold type, with the local amino acid residues also indicated:

1. Amino acid residues 105 to 113 of SEQ ID NO:2:

**LLNVDCDHY;**

15 2. Amino acid residues 324 to 332 of SEQ ID NO:2:

**SVTEDILTG; and**

3. Amino acid residues 362 to 374 of SEQ ID NO:2:

**DRLNQVLRWALGS.**

20 It must be noted that these invariable amino acids merely indicate that the T20782 derived amino acid sequence belongs to a very broad group of glycosyl transferases. Some of these enzymes such as cellulose synthase, chitin synthase, alginate synthase and hyaluronic acid synthase produce functionally very different compounds.

25 The presence of the conserved amino acid residues merely indicate that the T20782 clone may encode a  $\beta$ -glycosyl transferase protein such as the cellulose gene product, cellulose synthase. The fact that the clone localises in the vicinity of a gene involved in cellulose biosynthesis is the key feature which now focus interest on the T20782 clone as a candidate for the *RSW1* (cellulose synthase) gene.

30

- 53 -

The T20782 potentially codes for a cellulose synthase.

#### EXAMPLE 5

##### 5 NUCLEOTIDE SEQUENCE ANALYSIS OF THE GENOMIC CLONE 23H12

Clone 23H12 contains approximately 21kb of *Arabidopsis thaliana* genomic DNA in the region between the left border and right border T-DNA sequences, and localises to the RSW1 candidate YAC yUP5C8. Clone 23H12 was isolated by hybridisation using EST20782  
10 insert DNA, from a genomic DNA library made for plant transformation. Cosmid 12C4 was also shown to hybridize to the cDNA clone T20782, however this cosmid appears to comprise a partial genomic sequence corresponding to the related *Ath-A* cDNA sequence set forth in SEQ ID NO:7, for which the corresponding amino acid sequence is set forth in SEQ ID NO:8.

15

A restriction enzyme map of clone 23H12 is presented in Figure 5.

Nucleotide sequence of 8411bp of genomic DNA in the binary cosmid clone 23H12 was obtained (SEQ ID NO:3) by primer walking along the 23H12 template, using a Dye  
20 Terminator Cycle Sequencing kit (Perkin Elmer cat. #401384) as recommended by the manufacturer. The following primers at least, were used for DNA sequencing of the 23H12 clone DNA:

- a)cs1-R 5'-CAATGCATTAGCTCCAGCCT-3'
- 25 b)cs1-F 5'-AAAAGGCTGGAGCTATGAATGCAT-3'
- c)up 5'-AGAACAGCAGATAACACGGA-3'
- d)ve76-R2 5'-ATCCGTGTATCTGCTGTTCTTACC-3'
- e)est1-R 5'-AATGCTCTTGTGCCAAAGCAC-3'
- f)sve76-F 5'-ATTGTCCAGCCTTCTTCAGG-3'
- 30 g)ve76-R 5'-CTGAAGAAGGCTGGACAATGC-3'

- 54 -

h)B12-R1	5'-AGGTAAGCATAGCTGAACCATC-3'
i)B12-R2	5'-AGTAGATTGCAGATGGTTTCTAC-3'
j)B12-R3	5'-TTCAATGGGTCCACTGTACTAAC-3'
k)B12-R4	5'-ATTCAGATGCACCATTGTC-3'

5

The structure of the *RSW1* gene contained in cosmid clone 23H12 is also presented in Figure 5. As shown therein, coding sequences in 23H12, from the last 12 bp of exon 7 to the end of exon 14, correspond to the full T20782 cDNA sequence (i.e. SEQ ID NO:1). The nucleotide sequences of the *RSW1* gene comprising exons 1 to 8 were amplified from 10 *A.thaliana* Columbia double-stranded cDNA, using amplification primers upstream of the *RSW1* start site and a primer internal to the EST clone T20782.

The exons in the *RSW1* gene range from 81bp to 585bp in length and all 5' and 3' intron/exon splice junctions conform to the conserved intron rule.

15

The *RSW1* transcript comprises a 5'-untranslated sequence of at least 70bp in length, a 3243bp coding region and a 360bp 3'-untranslated region. Northern hybridization analyses indicate that the *RSW1* transcript in wild-type *A. thaliana* roots, leaves and inflorescences is approximately 4.0kb in length, and that a similar transcript size occurs in mutant tissue 20 (data not shown).

The derived amino acid sequence of the *RSW1* polypeptide encoded by the cosmid clone 23H12 (i.e. the polypeptide set forth in SEQ ID NO:6) is 1081 amino acids in length and contains the entire D, D, D, QXXRW signature characteristic of  $\beta$ -glycosyl transferase 25 proteins, between amino acid position 395 and amino acid position 822. The conserved Aspartate, Glutamine, Arginine and Tryptophan residues are shown below, in bold type, with the local amino acid residues also indicated:

1. amino acid residues 391 to 399 of SEQ ID NO:6:

30

YVSDDGSAM

- 55 -

2. Amino acid residues 557 to 565 of SEQ ID NO:6:

LLNVDCDHY;

3. Amino acid residues 776 to 784 of SEQ ID NO:6:

SVTEDILTG; and

5 4. Amino acid residues 814 to 826 of SEQ ID NO:6:

DRLNQVLRWALGS.

The second and third conserved Aspartate residues listed *supra*, and the fourth conserved amino acid sequence motif listed *supra* (i.e. QVLRW) are also present in the cDNA clone  
10 T20782 (see Example 4 above).

The 23H12 clone potentially encodes a cellulose synthase.

15

## EXAMPLE 6

### COMPLEMENTATION OF THE *rsw1* MUTATION

The complementation of the cellulose mutant plant *rsw1* is the key test to demonstrate the function of the clone 23H12 gene product. Complementation of the *rsw1* phenotype was  
20 demonstrated by transforming the binary cosmid clone 23H12, or a derivative clone thereof encoding a functional gene product, into the *Arabidopsis thaliana* cellulose mutant *rsw1*. Two DNA constructs (23H12 and pRSW1) were used to complement the *rsw1* mutant plant line.

#### 25 1. Construct 23H12

Clone 23H12 is described in Example 5 and Figure 5.

#### 2. Construct pRSW1

The 23H12 construct has an insert of about 21kb in length. To demonstrate that any  
30 complementation of the phenotype of the *rsw1* mutation is the result of expression of the gene

- 56 -

which corresponds to SEQ ID NO:3, a genetic construct, designated as pRSW1, comprising the putative RSW1 gene with most of the surrounding DNA deleted, was produced. A restriction enzyme (RE) map of the *RSW1* gene insert in pRSW1 is provided in Figure 5.

5 To produce pRSW1, the RSW1 gene was subcloned from cosmid 23H12 and cloned into the binary plasmid pBIN19. Briefly, *Escherichia coli* cells containing cosmid 23H12 were grown in LB medium supplemented with tetracyclin (3.5 mg/L). Plasmid DNA was prepared by alkaline lysis and digested sequentially with restriction enzymes *Pvu*II and *Sal*I. Two co-migrating fragments of 9 kb and 10 kb, respectively, were isolated as a single fraction from  
10 a 0.8% (w/v) agarose gel. The RSW1 gene was contained on the 10 kb *Pvu*II/*Sal*I fragment. The 9 kb fragment appeared to be a *Pvu*II cleavage product not comprising the RSW1 gene. The restriction fragments were ligated into pBIN19 digested with *Sma*I and *Sal*I. An aliquot of the ligation mix was introduced by electroporation into *E.coli* strain XLB1. Colonies resistant to kanamycin (50 mg/L) were selected and subsequently characterised by restriction  
15 enzyme analysis to identify those clones which contained only the 10 kb *Pvu*II/*Sal*I fragment comprising the RSW1 gene, in pBIN19.

### 3. Transfer of the 23H12 and pRSW1 constructs to *Agrobacterium tumefaciens*

Cosmid 23H12 was transferred to Agrobacterium by triparental mating, essentially as  
20 described by Ditta *et al.* (1980). Three bacterial strains as follows were mixed on solid LB medium without antibiotics: Strain 1 was an *E. coli* helper strain containing the mobilising plasmid pRK2013, grown to stationary phase; Strain 2 was *E.coli* containing cosmid 23H12, grown to stationary phase; and Strain 3 was an exponential-phase culture of *A. tumefaciens* strain AGL1 (Lazo *et al.*, 1991). The mixture was allowed to grow over night at 28°C, before  
25 an aliquot was streaked out on solid LB medium containing antibiotics (ampicillin 50 mg/L, rifampicin 50 mg/L, tetracyclin 3.5 mg/L) to select for transformed *A. tumefaciens* AGL1. Resistant colonies appeared after 2-3 days at 28°C and were streaked out once again on selective medium for further purification. Selected colonies were then subcultured in liquid LB medium supplemented with rifampicin (50 mg/L) and tetracyclin (3.5 mg/L) and stored at  
30 -80°C.

- 57 -

Plasmid pRSW1 (initially designated as p2029) was introduced into *A. tumefaciens* strain AGL1 by electroporation.

#### 4. Transformation of *rsw1* plants

5 The *rsw1* plant line was transformed with constructs 23H12 and pRSW1 using vacuum infiltration essentially as described by Bechtold et al. (1993).

#### 5. Analysis of radial swelling in transformants

Complementation of the radial swelling (*rsw*) phenotype , which is characteristic of the *rsw1* 10 mutant plant, was assayed by germinating transformed (i.e. T1 seed) *rsw1* seeds obtained as described *supra* on Hoaglands plates containing 50 $\mu$ g/ml kanamycin. Plates containing the transformed seeds were incubated at 21 °C for 10-12 days. Kanamycin-resistant seedlings were transferred to fresh Hoaglands plates containing 50 $\mu$ g/ml kanamycin and incubated at 31 °C for 2 days . Following this incubation, the root tip was examined for a radial swelling 15 phenotype. Under these conditions, the roots of wild-type plants do not show any radial swelling phenotype however, the roots of *rsw1* plants show clear radial swelling at the root tip and also have a short root compared to the wild-type plants. As a consequence, determination of the radial swelling phenotype of the transformed plants was indicative of successful complementation of the *rsw1* phenotype.

20

The kanamycin-resistant seedlings were maintained by further growth of seedlings at 21 °C, following the high temperature incubation. Once plants had recovered, the seedlings were transferred to soil and grown in cabinets at 21 °C (16 hr light/8 hr dark cycle). T2 seed was then harvested from mature individual plants.

25

Using the 23H12 construct for *rsw1* transformation, a total of 262 kanamycin-resistant seedlings were obtained. All of these transformants were tested for complementation of the root radial swelling phenotype. A total of 230 seedlings showed a wild type root phenotype, while only 32 seedlings showed the radial swelling root phenotype characteristic of *rsw1* 30 plants. By way of example, Figure 6 shows the phenotypes of transformed seedlings compared

- 58 -

to untransformed wild-type and *rsw1* seedlings, following incubation at 31°C. As shown in Figure 6, there is clear complementation of the radial swelling phenotype in the transformed seedlings, with normal root length being exhibited by the transformed seedlings at 31°C.

5 Using the pRSW1 construct for transformation, a total of 140 kanamycin-resistant seedlings were obtained. All of the 11 seedlings tested for complementation of the root radial swelling phenotype showed a wild type root phenotype and none of the seedlings showed any signs of radial swelling in the roots (data not shown).

10 **6. General morphological analysis of the complemented *rsw1* mutant line**

Further characterisation of the complemented *rsw1* plants has shown that other morphological characteristics of *rsw1* have also been restored in the transgenic lines, for example the bolt (inflorescence) height, and the ability of the plants to grow wild type cotyledons, leaves, trichomes, siliques and flowers at 31°C (data not shown).

15

**7. Biochemical complementation of the *rsw1* mutant line**

T2 seed from transformations using cosmid 23H12 as described *supra* or alternatively, using the binary plasmid pBin19 which lacks any *RSW1* gene sequences, was sown on Hoagland's solid media containing kanamycin (50µg/ml), incubated for 2 days at 21°C and then 20 transferred to 31°C for 5 days. Wild-type *A.thaliana* Columbia plants were grown under similar conditions but without kanamycin in the growth medium. Kanamycin resistant T2 seedlings which have at least one copy of the 23H12 cosmid sequence, and wild-type seedlings, were collected and frozen for cellulose analysis.

25 Cellulose levels were determined as acetic-nitric acid insoluble material (Updegraph, 1969) for 10 lines of kanamycin-resistant T2 plants transformed with the 23H12 cosmid sequence, and compared to the cellulose levels in *rsw1* mutant plants, wild-type *A.thaliana* Columbia plants and *A.thaliana* Columbia plants transformed with the binary plasmid pBin19. The results are provided in Table 7.

30

- 59 -

As shown in Table 7, the cellulose levels have been significantly elevated in the complemented *rsw1* (T2) plants, compared to the cellulose levels measured in the *rsw1* mutant parent plant. In fact, cellulose levels in the 23H12-transformed plants, expressed relative to the fresh weight of plant material or on a per seedling basis, are not significantly different from 5 the cellulose levels of either wild-type *Arabidopsis thaliana* Columbia plants or *A.thaliana* Columbia transformed with the binary plasmid pBin19. These data indicate that the 23H12 cosmid is able to fully complement the cellulose-deficient phenotype of the *rsw1* mutant.

Homozygous T3 lines are generated to confirm the data presented in Table 7.

10

Furthermore, data presented in Table 7 indicate that there is no difference in the rate of growth of the T2 transformed *rsw1* plants and wild-type plants at 31°C, because the fresh weight of such plants does not differ significantly. In contrast, the fresh weight of mutant *rsw1* seedlings grown under identical conditions is only approximately 55% of the level 15 observed in T2 lines transformed with 23H12 (range about 30% to about 80%). These data support the conclusion that cellulose levels have been manipulated in the complemented *rsw1* (T2) plants.

Furthermore, the rate of cellulose synthesis in 23H12-transformed plants and wild-type 20 plants at 31°C, as measured by <sup>14</sup>C incorporation is also determined.

Furthermore, the β-1,4-glucan levels and starch levels in the 23H12 transformant lines are shown to be similar to the β-1,4-glucan and starch levels in wild-type plants.

25

- 60 -

**TABLE 7**  
**CELLULOSE LEVELS IN *rsw1* PLANTS TRANSFORMED**  
**WITH COSMID CLONE 23H12**

5

PLANT LINE	SAMPLE SIZE (No. of plants)	SEEDLING FRESH WEIGHT (mg)	CELLULOSE (mg cellulose/ 100 mg tissue)	CELLULOSE (mg cellulose/ seedling)
1.2 ( <i>rsw1</i> +23H12)	126	2.51	1.23	0.031
10	1.4 ( <i>rsw1</i> +23H12)	132	2.25	2.50
	2.1 ( <i>rsw1</i> +23H12)	126	3.23	1.29
	3.1 ( <i>rsw1</i> +23H12)	127	3.75	1.23
	3.10 ( <i>rsw1</i> +23H12)	128	3.52	1.69
	4.4 ( <i>rsw1</i> +23H12)	110	5.14	1.31
15	4.5 ( <i>rsw1</i> +23H12)	125	3.18	1.26
	5.3 ( <i>rsw1</i> +23H12)	124	2.77	1.17
	9.2 ( <i>rsw1</i> +23H12)	125	2.26	1.41
	10.8 ( <i>rsw1</i> +23H12)	126	2.4	1.20
	Columbia/pBin19	106	2.64	1.34
20	Columbia	178	2.73	1.18
	<i>rsw1</i> mutant	179	1.77	0.84
				0.015

25

- 61 -

#### EXAMPLE 7

#### DETERMINATION OF THE FULL-LENGTH NUCLEOTIDE SEQUENCE ENCODING THE WILD-TYPE RSW1 POLYPEPTIDE

5 *Arabidopsis thaliana* double-stranded cDNA and cDNA libraries were prepared using the CAPFINDER cDNA kit (Clontech). RNA was isolated from wild-type Columbia grown in sterile conditions for 21 days.

Approximately 100,000 cDNA clones in an unamplified cDNA library were screened under 10 standard hybridization conditions at 65°C, using a probe comprising <sup>32</sup>P-labelled DNA amplified from double stranded cDNA. To prepare the hybridization probe, the following amplification primers were used:

1. 2280-F: 5'GAATCGGCTACGAATTCTCCA 3'
2. 2370-F: 5'TTGGTTGCTGGATCCTACCGG 3'
- 15 3. csp1-R: 5'GGT TCT AAA TCT TCT TCC GTC 3'

wherein the primer combinations were either 2280-F/csp1-R or 2370-F/csp1-R. The primer 2280-F corresponds to nucleotide positions 2226 to 2246 in SEQ ID NO:3, upstream of the translation start site. The primer 2370-F corresponds to nucleotide positions 2314 to 2334 20 in SEQ ID NO:3, encoding amino acids 7 through 13 of the RSW1 polypeptide. The primer csp1-R comprises nucleotide sequences complementary to nucleotides 588 to 608 of the T20782 clone (SEQ ID NO:1) corresponding to nucleotides 6120 to 6140 of SEQ ID NO:3. The hybridization probes produced are approximately 1858 nucleotides in length (2280-F/csp1-R primer combination) or 1946 nucleotides in length (2370-F/csp1-R primer 25 combination).

Five hybridizing bacteriophage clones were identified, which were plaque-purified to homogeneity during two successive rounds of screening. Plasmids were rescued from the positively-hybridizing bacteriophage clones, using the Stratagene excision protocol for the 30 ZapExpress™ vector according to the manufacturer's instructions. Colony hybridizations

confirmed the identity of the clones.

Isolated cDNA clones were sequenced by primer walking similar to the method described in Examples 4 and 5 *supra*.

5

A full-length wild-type *RSW1* nucleotide sequence was compiled from the nucleotide sequences of two cDNA clones. First, the 3'-end of the cDNA, encoding amino acids 453-1081 of *RSW1*, corresponded to the nucleotide sequence of the EST clone T20782 (SEQ ID NO:1). The remaining cDNA sequence, encoding amino acids 1-654 of *RSW1*, was 10 generated by amplification of the 5'-end from cDNA, using primer 2280-F, which comprises nucleotide sequences approximately 50-70bp upstream of the *RSW1* translation start site in cosmid 23 H12, and primer csp1-R, which comprises nucleotide sequences complementary to nucleotides 588 to 608 of the T20782 clone (SEQ ID NO:1).

15 Several amplified clones are sequenced to show that no nucleotide errors were introduced by the amplification process. The 5' and 3' nucleotide sequences are spliced together to produce the complete *RSW1* open reading frame and 3'-untranslated region provided in SEQ ID NO:5.

20 Those skilled in the art will be aware that the 5'-end and 3'-end of the two incomplete cDNAs are spliced together to obtain a full-length cDNA clone, the nucleotide sequence of which is set forth in SEQ ID NO:5.

Of the remaining cDNA clones, no isolated cDNA clone comprised a nucleotide sequence 25 which precisely matched the nucleotide sequence of the *RSW1* gene present in cosmid 23H12. However, several clones containing closely-related sequences were obtained, as summarised in Table 8. The nucleotide sequences of the *Ath-A* and *Ath-B* cDNAs are provided herein as SEQ ID Nos: 7 and 9, respectively.

- 63 -

**TABLE 8**  
**CHARACTERISATION OF *A. thaliana* cDNA CLONES**

CLONE NAME	DESCRIPTION	LENGTH	SEQ ID NO:
RSW1.1A	chimeric clone	partial	not provided
5	RSW1A	chimeric clone	partial
	<i>Ath</i> -A	12C4 cDNA	full-length
	<i>Ath</i> -B	new sequence	full-length
	RSW4A	identical to <i>Ath</i> -B	full-length
			not provided

- 10 The derived amino acid sequences encoded by the cDNAs listed in Table 8, is provided in Figures 8 and 9 and SEQ ID Nos: 8 and 10 herein.

Figure 10 a schematic representation of the important features of the RSW1 polypeptide which are conserved within *A.thaliana* and between *A.thaliana* and other plant species. In 15 addition to the species indicated in Figure 10, the present inventors have also identified maize, wheat, barley and *Brassica* ssp. cellulose biosynthetic genes by homology search. Accordingly, the present invention extends to cellulose genes and cellulose biosynthetic polypeptides as hereinbefore defined, derived from any plant species, including *A. thaliana*, cotton, rice, wheat, barley, maize, *Eucalyptus* ssp., *Brassica* ssp., *Pinus* ssp., *Populus* ssp., 20 *Picea* ssp., hemp, jute and flax, amongst others.

#### EXAMPLE 8

#### ISOLATION OF FULL-LENGTH NUCLEOTIDE SEQUENCE ENCODING THE MUTANT RSW1 POLYPEPTIDE

25

*Arabidopsis thaliana* double-stranded cDNA and cDNA libraries were prepared using the CAPFINDER cDNA kit (Clontech). RNA was isolated from *Arabidopsis thaliana* Columbia *rsw1* mutant plants grown in sterile conditions for 21 days.

- 30 The full-length *rsw1* mutant nucleotide sequence was generated by sequencing two amplified

- 64 -

DNA fragments spanning the *rsw1* mutant gene. The 5'- end sequence of the cDNA (comprising the 5'-untranslated region and exons 1-11) was amplified using the primer combination 2280-F/csp1-R (Example 7). The 3'-end sequence was amplified using the primers EST1-F and cs3-R set forth below:

- 5        1. Primer EST1-F:                    5'AATGCTTCTTGTGCCAAAGCA 3'  
          2. Primer cs3-R:                    5'GACATGGAATCACCTTAAGTGC 3'

wherein primer EST1-F corresponds to nucleotide positions 1399-1420 of SEQ ID NO:5 (within exon 8) and primer cs3-R is complementary to nucleotides 3335-3359 of SEQ ID  
10 NO:5 (within the 3'-untranslated region of the wild-type transcript).

The full-length sequence of the mutant *rsw1* transcript is set forth herein as SEQ ID NO:11.

Whilst not being bound by any theory or mode of action, a single nucleotide substitution  
15 in the *rsw1* mutant nucleotide sequence (nucleotide position 1716 in SEQ ID NO:11),  
relative to the wild-type *RSW1* nucleotide sequence (nucleotide position 1646 in SEQ ID  
NO:5), resulting in Ala549 being substituted with Val549 in the mutant polypeptide, may  
contribute to the altered activity of the *RSW1* polypeptide at non-permissive temperatures  
such as 31°C. Additional amino acid substitutions are also contemplated by the present  
20 invention, to alter the activity of the *RSW1* polypeptide, or to make the polypeptide  
temperature-sensitive.

#### EXAMPLE 9

#### ANTISENSE INHIBITION OF CELLULOSE PRODUCTION IN TRANSGENIC PLANTS

##### 1. Construction of an antisense *RSW1* binary vector

One example of transgenic plants in which cellulose production is inhibited is provided by  
the expression of an antisense genetic construct therein. Antisense technology is used to  
30 target expression of a cellulose gene(s) to reduce the amount of cellulose produced by

- 65 -

transgenic plants.

By way of exemplification, an antisense plant transformation construct has been engineered to contain the T20782 cDNA insert (or a part thereof) in the antisense orientation and in 5 operable connection with the CaMV 35S promoter present in the binary plasmid pRD410 (Datla *et al.* 1992). More particularly, the T20782 cDNA clone, which comprises the 3'-end of the wild-type *RSW1* gene, was digested with *Xba*I and *Kpn*I and cloned into the kanamycin-resistant derivative of pGEM3zf(-), designated as plasmid, pJKKMf(-). The *RSW1* sequence was sub-cloned, in the antisense orientation, into the binary vector pRD410 10 as a *Xba*/I/*Sac*I fragment, thereby replacing the  $\beta$ -glucuronidase (GUS or *uidA*) gene. This allows the *RSW1* sequence to be transcribed in the antisense orientation under the control of the CaMV 35S promoter.

The antisense *RSW1* binary plasmid vector was transferred to *Agrobacterium tumefaciens* 15 strain AGL1, by triparental mating and selection on rifampicin and kanamycin, as described by Lazo *et al.* (1991). The presence of the *RSW1* insert in transformed *A.tumefaciens* cells was confirmed by Southern hybridization analysis (Southern, 1975). The construct was shown to be free of deletion or rearrangements prior to transformation of plant tissues, by back-transformation into *Escherichia coli* strain JM101 and restriction digestion analysis.

20

## 2. Transformation of *Arabidopsis thaliana*

Eight pots, each containing approximately 16 *A. thaliana* ecotype Columbia plants, were grown under standard conditions. Plant tissue was transformed with the antisense *RSW1* 25 binary plasmid by vacuum infiltration as described by Bechtold *et al* (1993). Infiltration media contained 2.5% (w/v) sucrose and plants were infiltrated for 2 min until a vacuum of approximately 400mm Hg was obtained. The vacuum connection was shut off and plants allowed to sit under vacuum for 5 min.

Approximately 34,000 T1 seed was screened on MS plates containing 50 $\mu$ g/ml kanamycin, 30 to select for plants containing the antisense *RSW1* construct. Of the T1 seed sown, 135

- 66 -

kanamycin-resistant seedlings were identified, of which 91 were transferred into soil and grown at 21 °C under a long-day photoperiod (16hr light; 8hr dark).

Of the 91 transgenic lines, 19 lines were chosen for further analysis which had 5 anther filaments in each flower which were too short to deposit pollen upon the stigma and, as a consequence, required hand-pollination to obtain T2 seed therefrom.

T2 seed from 14 of these 19 lines was plated out onto vertical Hoaglands plates containing kanamycin to determine segregation ratios. Between five and ten seed were plated per 10 transgenic line. Control seeds, including *A. thaliana* Columbia containing the binary vector pBIN19 (Bevan, 1984) and segregating 3:1 for kanamycin resistance, and the *rsw1* mutant transformed with the *NPTII* gene, also segregating 3:1 for kanamycin resistance, were grown under the same conditions. Kanamycin-resistant plants were transferred to soil and grown at 21 °C under long days, until flowering. Untransformed *Arabidopsis thaliana* Columbia plants 15 were also grown under similar conditions, in the absence of kanamycin.

### 3. Morphology of antisense- *RSW1* plants

A comparison of the morphology of antisense *RSP1* plants grown at 21 °C, to mutant *rsw1* plants grown at the non-permissive temperature (i.e. 31 °C) has identified a number of common 20 phenotypes. For example, the antisense plants exhibit reduced fertility, inflorescence shortening and have short anthers, compared to wild-type plants, when grown at 21 °C. These phenotypes are also observed in mutant *rsw1* plants grown at 31 °C. These results suggest that the antisense construct in the transgenic plants may be targeting the expression of the wild-type *RSP1* gene at 21 °C.

25

Figure 7 shows the reduced inflorescence (bolt) height in antisense 35S-*RSP1* plants compared to wild-type *A. thaliana* Columbia plants grown under identical conditions.

### 4. Cell wall carbohydrate analysis of antisense plants.

30 T3 plants which are homozygous for the 35S-*RSP1* antisense construct are generated and the

content of cellulose therein is determined as described in Example 1. Plants expressing the antisense construct are shown to have significantly less cellulose in their cell walls, compared to wild-type plants. Additionally, the levels of non-crystalline  $\beta$ -1,4-glucan and starch are elevated in the cells of antisense plants, compared to otherwise isogenic plant lines which have  
5 not been transformed with the antisense genetic construct.

##### 5. Antisense 35S-RSW1 mRNA expression levels in transgenic plants

Total RNA was extracted from 0.2g of leaf tissue derived from 33 kanamycin-resistant T1 plants containing the antisense 35S-RSW1 genetic construct, essentially according to  
10 Longemann *et al.* (1986). Total RNA (25  $\mu$ g) was separated on a 2.2M formaldehyde/agarose gel, blotted onto nylon filters and hybridized to a riboprobe comprising the sense strand sequence of the cDNA clone T20782. To produce the riboprobe, T7 RNA polymerase was used to transcribe sense RNA from a linearised plasmid template containing T20782, in the presence of [ $\alpha$ - $^{32}$ P]UTP. Hybridizations and subsequent washes were performed as described  
15 by Dolferus *et al.* (1994). Hybridized membranes were exposed to Phosphor screens (Molecular Dynamics, USA).

The levels of expression of the RSW1 antisense transcript were determined and compared to the level of fertility observed for the plant lines. As shown in Table 9, the level of antisense  
20 gene expression is correlated with the reduced fertility phenotype of the antisense plants. In 13 lines, a very high or high level of expression of the 35S-RSW1 antisense gene was observed and, in 11 of these lines fertility was reduced. Only lines 2W and 3E which expressed high to very high levels of antisense mRNA, appeared to be fully fertile. In 12 lines which expressed medium levels of antisense mRNA, approximately one-half were fertile and one-half appeared  
25 to exhibit reduced fertility. In contrast, in 8 plant lines in which only a low or very low level of expression of the antisense 35S-RSW1 genetic construct was observed, a wild-type (i.e. fertile) phenotype was observed for all but one transgenic line, line 2R.

Data presented in Table 9 and Figure 7 indicate that the phenotype of the cellulose-deficient  
30 mutant *rsw1* may be reproduced by expressing antisense *RSW1* genetic constructs in transgenic

plants.

To confirm reduced cellulose synthesis and/or deposition in transgenic plants expressing the antisense *RSW1* gene, the level of cellulose is measured by the  $^{14}\text{C}$  incorporation assay or 5 as acetic/nitric acid insoluble material as described in Example 1 and compared to cellulose production in otherwise isogenic wild-type plants. Cellulose production in the transgenic plants is shown to be significantly reduced compared to wild-type plants. The severity of phenotype of the transgenic plants thus produced varies considerably, depending to some extent upon the level of inhibition of cellulose biosynthesis.

10

**TABLE 9**

- 69 -

**LEVELS OF ANTISENSE GENE EXPRESSION AND FERTILITY IN  
T1 LINES OF ANTISENSE 35S-RSW1 PLANTS**

5	T1 PLANT LINE	ANTISENSE 35S-RSW1 EXPRESSION	FERTILITY	T1 PLANT LINE	ANTISENSE 35S-RSW1 EXPRESSION	FERTILITY
B	very high	sterile*		2H	medium	fertile
2B	very high	sterile*		C	medium	sterile*
3E	very high	fertile		F	medium	sterile*
10	2E	high	sterile*	2Q	medium	fertile
2K	high	sterile*		3P	medium	sterile*
2M	high	sterile*		3T	medium	fertile
2O	high	sterile*		5D	medium	sterile*
15	2P	high	sterile*	6A	medium	fertile
2W	high	fertile		8E	low	fertile
2Z	high	sterile*		2R	low	sterile*
3G	high	sterile*		7A	low	fertile
3Q	high	sterile*		7S	low	fertile
20	7Q	high	sterile*	7O	low	fertile
7N	medium	sterile*		7R	low	fertile
7G	medium	fertile		1B	very low	fertile
1C	medium	sterile*		2U	very low	fertile
2X	medium	sterile*				

\*sterile phenotype not indicative of complete sterility, but that hand pollination at least, is

25 required to obtain seed from such plants.

- 70 -

**EXAMPLE 10**  
**RSW1 RELATED SEQUENCES IN RICE PLANTS**

To identify *RSW1* related nucleotide sequences in rice, a genetic sequence database was  
5 searched for nucleotide sequences which were closely-related to one or more of the  
*Arabidopsis thaliana* *RSW1* nucleotide sequences described in the preceding Examples. Rice  
EST S0542 (MAFF DNA bank, Japan) was identified, for which only a partial nucleotide  
sequences was available. Additionally, before the instant invention, there was no probable  
function attached to the rice EST S0542 sequence.

10

The present inventors have obtained the complete nucleotide sequence of clone S0542 and  
derived the amino acid sequence encoded therefor. The S0542 cDNA is only 1741bp in length  
and appears to be a partial cDNA clone because, although it comprises 100bp of 5'-  
untranslated sequence and contains the ATG start codon, it is truncated at 3'-end and, as a  
15 consequence encodes only the first 547 amino acid residues of the rice *RSW1* or *RSW1*-like  
polypeptide. Based upon the length of the corresponding *Arabidopsis thaliana* *RSW1*  
polypeptide (1081 amino acids), the rice *RSW1* sequence set forth in SEQ ID NO:14 appears  
to contain approximately one-half of the complete amino acid sequence.

20 The N-terminal half of the rice *RSW1* amino acid sequence is approximately 70% identical to  
the *Arabidopsis thaliana* *RSW1* polypeptide set forth in SEQ ID NO:6, with higher homology  
(approximately 90%) occurring between amino acid residues 271-547 of the rice sequence.  
These data strongly suggest that S0542 is the rice homologue of the *A. thaliana* *RSW1* gene.  
Alignments of rice, *A. thaliana* and cotton *RSW1* amino acid sequences are presented in  
25 Figures 9 and 10.

To isolate full-length cDNA clones and genomic clone equivalents of S0542 (this study and  
MAFF DNA bank, Japan) or D48636 (Pear *et al.*, 1996), cDNA and genomic clone libraries  
are produced using rice mRNA and genomic DNA respectively, and screened by hybridisation  
30 using the S0542 or D48636 cDNAs as a probe, essentially as described herein. Positive-

- 71 -

hybridising plaques are identified and plaque-purified, during further rounds of screening by hybridisation, to single plaques.

The rice clones are sequenced as described in the preceding Examples to determine the 5 complete nucleotide sequences of the rice *RSW1* genes and derived amino acid sequences therefor. Those skilled in the art will be aware that such gene sequences are useful for the production of transgenic plants, in particular transgenic cereal plants having altered cellulose content and/or quality, using standard techniques. The present invention extends to all such genetic sequences and applications therefor.

10

#### **EXAMPLE 11**

#### ***RSW1* RELATED SEQUENCES IN COTTON PLANTS**

15 A <sup>32</sup>P-labelled *RSW1* PCR fragment was used to screen approximately 200,000 cDNA clones in a cotton fibre cDNA library. The *RSW1* PCR probe was initially amplified from *Arabidopsis thaliana* wild type cDNA using the primers 2280-F and csp1-R described in the preceding Examples, and then re-amplified using the primer combination 2370-F/csp1-R, also described in the preceding Examples.

20

Hybridisations were carried out under low stringency conditions at 55°C.

Six putative positive-hybridising plaques were identified in the first screening round. Using two further rounds of screening by hybridisation, four of these plaques were purified to single 25 plaques. Three plaques hybridise very strongly to the *RSW1* probe while the fourth plaque hybridises less intensely.

We conclude that the positive-hybridising plaques which have been purified are strong candidates for comprising cotton *RSW1* gene sequences or *RSW1*-like gene sequences.

30 Furthermore, the cotton cDNAs may encode the catalytic subunit of cellulose synthase,

because the subunit protein architecture of cellulose synthase appears to be highly conserved among plants as highlighted in the preceding Example.

Furthermore, a Southern blot of cotton genomic DNA digested with *Bg*/II was hybridised with 5 the 5' end of the *RSW1* cDNA, under low stringency hybridisation conditions at 55°C. Results are presented in Figure 11. These data demonstrate that *RSW1*-related sequences exist in the cotton genome.

The cotton cDNA clones described herein are sequenced as described in the preceding 10 Examples and used to produce transgenic cotton plants having altered fibre characteristics. The cDNAs are also used to genetically alter the cellulose content and/or quality of other plants, using standard techniques.

#### EXAMPLE 12

##### 15 ***RSW1 RELATED SEQUENCES IN EUCALYPTUS SSP.***

Putative *Eucalyptus* ssp. cellulose synthase catalytic subunit gene fragments were obtained by amplification using PCR. DNA primers were designed to conserved amino acid residues found in the *Arabidopsis thaliana RSW1* and 12C4 amino acid sequences. Three primers were used 20 for PCR. The primers are listed below:

pcsF-I 5'- A A/G A A G A T I G A C/T T A C/T C/T T I A A A/G G A C/T A A-3'

pcsR-II 5'-A T I G T I G G I G T I C G/T A/G T T C/T T G A/T/G/C C T/G A/T/C/G C C -3'

pcsF-II 5'- G C I A T G A A A/G A/C G I G A I T A C/T G A A/G G A -3'

25

Using standard PCR conditions (50°C annealing temperature) and solutions, the primer sets pcsF-I/pcsR-II and pcsF-II/pcsR-II were used to amplify genetic sequences from pooled *Eucalyptus* ssp. cDNA. In the first reaction primers pcsF-I and pcsR-II were used to generate a fragment approximately 700 bp in length. In the second PCR reaction, which used primers 30 pcsF-II and pcsR-II, a fragment estimated to 700 bp was obtained. The sizes of the PCR

fragments are within the size range estimated for the corresponding *Arabidopsis thaliana* sequences.

We conclude that the amplified *Eucalyptus* ssp. PCR fragments are likely to be related to the 5 *Arabidopsis thaliana RSW1* gene and may encode at least a part of the *Eucalyptus* ssp. cellulose synthase catalytic subunit.

The *Eucalyptus* ssp. PCR clones described herein are sequenced as described in the preceding Examples and used to isolate the corresponding full-length *Eucalyptus* ssp cDNAs and 10 genomic gene equivalents. Those skilled in the art will be aware that such gene sequences are useful for the production of transgenic plants, in particular transgenic *Eucalyptus* ssp plants having altered cellulose content and/or quality, using standard techniques. The present invention extends to all such genetic sequences and applications therefor.

15

#### EXAMPLE 13

##### "NON-CRYSTALLINE $\beta$ -1,4-GLUCAN AS A MODIFIER"

##### OF CELL WALL PROPERTIES

20 The properties of plant cell walls depend on the carbohydrates, proteins and other polymers of which they are composed and the complex ways in which they interact. Increasing the quantities of non-crystalline  $\beta$ -1,4-glucan in cell walls affects those wall properties which influence mechanical, nutritional and many other qualities as well as having secondary consequences resulting from the diversion of carbon into non-crystalline glucan at the expense 25 of other uses. To illustrate one of these effects, we investigated the ability of the non-crystalline glucan to hydrogen bond to other wall components particularly cellulose in the way that has been shown to be important for wall mechanics.

Hemicelluloses such as xyloglucans cross-link cellulose microfibrils by hydrogen bonding to 30 the microfibril surface (Levy *et al.*, 1991). Since the  $\beta$ -1,4-glucan backbone of xyloglucan is

thought to be responsible for hydrogen bonding (with the xylose, galactose and fucose substitutions limiting the capacity to form further hydrogen bonds) we can expect the non-crystalline  $\beta$ -1,4-glucan also to have a capacity to hydrogen bond and cross link cellulose. The effectiveness of strong alkalis in extracting xyloglucans is thought to relate to their disruption 5 of the hydrogen bonds with cellulose (Hayashi and MacLachlan, 1984).

To demonstrate that the non-crystalline  $\beta$ -1,4-glucan forms similar associations with the cellulose microfibrils, we examined whether the 4 M KOH fraction, extracted from shoots of the *rsw1* mutant and from wild type *RSW1* plants, contained non-crystalline glucan in addition 10 to xyloglucan. The non-crystalline glucan was separated from xyloglucan in the 4 M KOH extract by dialysing the neutralised extract against distilled water and centrifuging at 14000 g for 1 hour. The pellet was shown to be a pure  $\beta$ -1,4-glucan by using the methods for monosaccharide analysis, methylation analysis and enzyme digestion used to characterise the glucan in the ammonium oxalate fraction (see Example 1).

15

Table 10 shows the presence of substantial quantities of glucan recovered in pure form in the pellet from 4 M KOH fractions extracted from the overproducing *rsw1* mutant of *Arabidopsis thaliana*. These data also demonstrate the presence of smaller quantities of non-crystalline  $\beta$ -1,4-glucan in the 4 M KOH fraction from wild type plants, compared to *rsw1*, particularly 20 when grown at 31 °C.

**TABLE 10**  
Glucose contents\* of 4M KOH fractions from shoots of wild-type and  
*rsw1* mutant *Arabidopsis thaliana* plants

Glucose fraction	wild-type		<i>rsw1</i> mutant	
	21 °C	31 °C	21 °C	31 °C
xyloglucan and non-crystalline glucan in whole extract	36.4	56.9	27.1	93.1
non-crystalline glucan in pellet	7.8	20.5	7.6	56.0

\* nmol glucose/ mg plant dry weight after TFA hydrolysis

- 75 -

The monosaccharide composition of the supernatant remaining after centrifugation was determined after TFA hydrolysis. These data, and data from methylation analysis, are consistent with the supernatant being a relatively pure xyloglucan. The supernatant was free of glucan, because no glucose could be released by the endocellulase/β-glucosidase mixture  
5 that released glucose from β-1,4-glucan.

The presence of both non-crystalline β-1,4-glucan and xyloglucan in the 4 M KOH fraction, when taken together with the implications from structural predictions (Levy *et al*, 1991), is consistent with some of the non-crystalline β-1,4-glucan in the wall hydrogen bonding to  
10 cellulose microfibrils in similar fashion to the β-1,4-glucan backbone of xyloglucan.

The cross linking provided when xyloglucans and other hemicelluloses bind to two or more microfibrils is an important determinant of the mechanical properties of cellulosic walls (Hayashi, 1989). The effects of increasing the amounts of non-crystalline β-1,4-glucan in walls  
15 are likely to be greatest in walls which otherwise possess relatively low levels of cross linking as a result of high ratios of cellulose: hemicelluloses. Such conditions are common in secondary walls including those of various fibres, and the cellulose:hemicellulose ratio is particularly high in cotton fibres.

20 The effects on wall mechanical properties of overproducing non-crystalline glucan are shown by transforming plants with the mutant allele of *rsw1* (SEQ ID NO:11) operably under the control of either the *RSW1* promoter derived from SEQ ID NO:3 or SEQ ID NO:4 or alternatively, an appropriate constitutive promoter such as the CaMV 35S promoter. Production of non-crystalline glucan is quantified by fractionating the cell walls using the  
25 methods described above to show in particular that non-crystalline glucan is recovered in the 4 M KOH fraction. Mechanical properties of the cell walls are measured using standard methods for fibre analysis to study parameters such as stress-strain curves, and breaking strain, amongst other properties.

- 76 -

**EXAMPLE 14**  
**OVER-EXPRESSION OF CELLULOSE SYNTHASE**  
**IN TRANSGENIC PLANTS**

5 Three strategies are employed to over-express cellulose synthase in *Arabidopsis thaliana* plants.

In the first strategy, the CaMV 35S promoter sequence is operably connected to the full-length cellulose synthase cDNA which is obtainable by primer extension of SEQ ID NO:1. This is  
10 achievable by cloning the full-length cDNA encoding cellulose synthase, in the sense orientation, between the CaMV 35S promoter or other suitable promoter operable in plants and the nopaline synthase terminator sequences of the binary plasmid pBI121.

In the second strategy, the coding part of the genomic gene is cloned, in the sense orientation,  
15 between the CaMV 35S promoter and the nopaline synthase terminator sequences of the binary plasmid pBI121.

In the third strategy, the 23H12 binary cosmid clone or the derivative pRSW1, containing the cellulose synthase gene sequence operably under the control of the cellulose synthase gene  
20 promoter and terminator sequences is prepared in a form suitable for transformation of plant tissue.

For *Agrobacterium*-mediated tissue transformation, binary plasmid constructs discussed *supra* are transformed into *Agrobacterium tumefaciens* strain AGL1 or other suitable strain. The  
25 recombinant DNA constructs are then introduced into wild type *Arabidopsis thaliana* plants (Columbia ecotype), as described in the preceding Examples.

Alternatively, plant tissue is directly transformed using the vacuum infiltration method described by Beshtold *et al.* (1993).

- 77 -

The transgenic plants thus produced exhibit a range of phenotypes, partly because of position effects and variable levels of expression of the cellulose synthase transgene.

Cellulose content in the transgenic plants and isogenic untransformed control plants is 5 determined by the  $^{14}\text{C}$  incorporation assay or as acetic/nitric acid insoluble material as described in Example 1. In general, the level of cellulose deposition and rates of cellulose biosynthesis in the transgenic plants are significantly greater than for untransformed control plants.

10 Furthermore, in some cases, co-suppression leads to mimicry of the *rsw1* mutant phenotype.

**EXAMPLE 15**  
**SITE-DIRECTED MUTAGENESIS OF THE *RSW1* GENE**

15

The nucleotide sequence of the *RSW1* gene contained in 23H12 is mutated using site-directed mutagenesis, at several positions to alter its catalytic activity or substrate affinity or glucan properties. In one example, the *RSW1* gene is mutated to comprise one or more mutations present in the mutant *rsw1* allele.

20

The mutated genetic sequences are cloned into binary plasmid described in the preceding Examples, in place of the wild-type sequences. Plant tissue obtained from both wild-type *Arabidopsis thaliana* (Columbia) plants and *A. thaliana rsw1* plants is transformed as described herein and whole plants are regenerated.

25

Control transformations are performed using the wild-type cellulose synthase gene sequence.

**EXAMPLE 16****PHENOTYPES OF PLANTS EXPRESSING MUTATED *RSW1* GENES**

Plants transformed with genetic constructs described in Example 15 (and elsewhere) are 5 categorised initially on the basis of number of transgene copies, to eliminate variability arising therefrom. Plants expressing single copies of different transgenes are analysed further for cell wall components, including cellulose, non-crystalline  $\beta$ -1,4-glucan polymer, starch and carbohydrate content.

**10 1. Cellulose content**

Cellulose content in the transgenic plants is determined by the  $^{14}\text{C}$  incorporation assay as described in Example 1. Cell walls are prepared, fractionated and the monosaccharide composition of individual fractions determined as in Example 1.

**15 2. Non-crystalline  $\beta$ -1,4-glucan content**

Transgenic plants expressing the *rsw1* mutant allele exhibit a higher level of non-crystalline, and therefore extractable,  $\beta$ -1,4-glucan in cell walls compared to plants expressing an additional copy of the wild-type *RSW1* allele. Thus, it is possible to change the crystallinity of the  $\beta$ -1,4-glucan chains present in the cell wall by mutation of the wild-type *RSW1* allele.

20

**3. Starch content**

Transgenic plants are also analysed to determine the effect of mutagenesis of the *RSW1* gene on the level of starch deposited in their roots. The quantity of starch present in material prepared from the crude wall fraction is determined using the anthrone/ $\text{H}_2\text{SO}_4$  method 25 described in Example 1. The data show that mutating the *RSW1* gene to the mutant *rsw1* allele increases starch deposition. This demonstrates that the gene can be used to alter the partitioning of carbon into carbohydrates other than cellulose.

**4. Cell wall composition**

30 The cell wall composition of transgenic plant material is also analysed. Wild type and *rsw1*

- 79 -

and transgenic seedlings are grown for 2 d at 21 °C and then kept for a further 5 d at either 21 °C or 31 °C. With transfer to 31 °C when the seed has scarcely germinated, the wall composition at final harvest largely reflects the operation of the mutated *rsw1* gene product at its restrictive temperature. Cell wall fractionation is carried out in similar fashion to that 5 described for the <sup>14</sup>C-experiment (Example 1) and the monosaccharide composition of each fraction is quantified by GC/MS after hydrolysis with trifluoroacetic acid or, in the case of crystalline cellulose, H<sub>2</sub>SO<sub>4</sub>.

In some transgenic plants in which the *RSW1* gene is mutated, the monosaccharide 10 composition is comparable to that observed for homozygous *rsw1* plants, at least in some cases, confirming that there is a major reduction in the quantity of crystalline cellulose in the final, acid insoluble fraction. Thus, mutation of the *RSW1* gene can be performed to produce changes in the composition of plant cell walls.

15

#### EXAMPLE 17

##### **CHEMICAL MODIFICATION OF THE *RSW1* GENE TO MANIPULATE CELLULOSE PRODUCTION AND PLANT CELL WALL CONTENT**

As demonstrated in the preceding Examples, the *RSW1* gene is involved in cellulose 20 production and the manipulation of cell wall content.

In the present Example, to identify novel phenotypes and gene sequences important for the normal functioning of the cellulose synthase gene, the *RSW1* gene is modified *in planta*, using the chemical mutagen EMS. The mutant plants are identified following germination and the 25 modified *RSW1* genes are isolated and characterised at the nucleotide sequence level. A sequence comparison between the mutant gene sequences and the wild type sequence reveals nucleotides which encode amino acids important to the normal catalytic activity of the cellulose synthase enzyme, at least in *Arabidopsis thaliana* plants.

30 This approach thus generates further gene sequences of utility in the modification of cellulose

- 80 -

content and properties in plants.

**EXAMPLE 18**  
**DISCUSSION**

5

Five pieces of evidence make a compelling case that the RSW1 gene product encodes the catalytic subunit of cellulose synthase:

1. The *rsw1* mutation selectively inhibits cellulose synthesis and promotes accumulation of a non-crystalline  $\beta$ -1,4-glucan;
  - 10 2. The *rsw1* mutation removes cellulose synthase complexes from the plasma membrane, providing a plausible mechanism for reduced cellulose accumulation and placing the RSW1 product either in the complexes or interacting with them;
  3. The D,D,D,QXXRW signature identifies the RSW1 gene product as a processive glycosyl transferase enzyme (Saxena, 1995);
  - 15 4. The wild type allele corrects the temperature sensitive phenotype of the *rsw1* mutant; and
  5. Antisense expression of the *RSW1* in transgenic plants grown at 21 °C reproduces some of the phenotype of *rsw1* which is observed following growth at 31 °C.
- 20 Consistent with the plasma membrane location expected for a catalytic subunit, the putative 122 kDa RSW1 product contains 8 predicted membrane-spanning regions. Six of these regions cluster near the C-terminus (Figure 10), separated from the other two by a domain that is probably cytoplasmic and has the weak sequence similarities to prokaryotic glycosyl transferases (Wong, 1990; Saxena, 1990 ; Matthyse, 1995; Sofia, 1994 ; Kutish, 1996).

25

RSW1 therefore qualifies as a member of the large family of *Arabidopsis thaliana* genes whose members show weak similarities to bacterial cellulose synthase. RSW1 is the first member of that family to be rigorously identified as an authentic cellulose synthase. Among the diverse genes in *A. thaliana*, at least two genes show very strong sequence similarities to 30 the *RSW1* gene and are most likely members of a highly conserved sub-family involved in

cellulose synthesis. The closely related sequences come from cosmid 12C4, a partial genomic clone cross-hybridising with EST T20782 designated *Ath*-A, and from a full length cDNA designated *Ath*-B.

5 *Ath*-A resembles *RSW1* (SEQ ID NO:5) at its N-terminus whereas *Ath*-B starts 22 amino acid residues downstream [Figure 8 and Figure 9(i), (ii) and (iii)]. Closely related sequences in other angiosperms are the rice EST S0542 [Figure 9(i), (ii) and (iii)], which resembles the polypeptides encoded by *RSW1* and *Ath*-A and the cotton *celA1* gene (Pear, 1996) at the N-terminus.

10

The *Arabidopsis thaliana*, rice and cotton genes have regions of very high sequence similarity interspersed with variable regions (Figures 9 and 10). Most of the highest conservation among those gene products occurs in their central cytoplasmic domain where the weak similarities to the bacterial cellulose synthase occur. The N-terminal region that precedes the first 15 membrane spanning region is probably also cytoplasmic but shows many amino acid substitutions as well as sequences in *RSW1* that have no counterpart in some of the other genes as already noted for *celA*. An exception to this is a region comprising 7 cysteine residues with highly conserved spacings (Figure 10). This is reminiscent of regions suggested to mediate protein-protein and protein-lipid interactions in diverse proteins including 20 transcriptional regulators and may account for the striking sequence similarity between this region of *RSW1* and two putative soybean bZIP transcription factors (Genbank SOYSTF1A and 1B).

In conclusion, the chemical and ultrastructural changes seen in the cellulose-deficient mutant 25 combine with gene cloning and complementation of the mutant to provide strong evidence that the *RSW1* locus encodes the catalytic subunit of cellulose synthase. Accumulation of non-crystalline  $\beta$ - 1,4-glucan in the shoot of the *rsw1* mutant suggests that properties affected by the mutation are required for glucan chains to assemble into microfibrils. Whilst not being bound by any theory or mode of action, a key property may be the aggregation of catalytic 30 subunits into plasma membrane rosettes. At the restrictive temperature, mutant synthase

complexes disassemble to monomers (or smaller oligomers) that are undetectable by freeze etching. At least in the shoot, the monomers seem to remain biosynthetically active but their  $\beta$ -1,4-glucan products fail to crystallise into microfibrils probably because the chains are growing from dispersed sites. Crystallisation into microfibrils, with all its consequences for  
5 wall mechanics and morphogenesis, therefore may depend upon catalytic subunits remaining aggregated as plasma membrane rosettes.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that  
10 the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

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- 84 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT: Australian National University and the Commonwealth Scientific  
and

Industrial Research Organisation

10 (ii) TITLE OF INVENTION: Manipulation of plant cellulose

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:

15

(A) ADDRESSEE: Davies Collison Cave Patent Attorneys

(B) STREET: 1, Little Collins Street

(C) CITY: Melbourne

(D) STATE: Victoria

(E) COUNTRY: Australia

20

(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

25

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

30

(A) APPLICATION NUMBER: PCT INTERNATIONAL

(B) FILING DATE:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: AU P00699

(B) FILING DATE: 27-JUN-1996

35

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: SLATTERY, JOHN M

(ix) TELECOMMUNICATION INFORMATION:

40

(A) TELEPHONE: 61-3-9254-2777

(B) TELEFAX: 61-3-9254-2770



- 86 -

ATA CAG GTG TTC TTA GGC CAT AGT GGG GGT CTG GAT ACC GAT GGA AAT Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly Asn 50                    55                    60	192
5 GAG CTG CCT AGA CTC ATC TAT GTT TCT CGT GAA AAG CGG CCT GGA TTT Glu Leu Pro Arg Leu Ile Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe 65                    70                    75                    80	240
CAA CAC CAC AAA AAG GCT GGA GCT ATG AAT GCA TCG ATC CGT GTA TCT 10 Gln His His Lys Lys Ala Gly Ala Met Asn Ala Ser Ile Arg Val Ser 85                    90                    95	288
GCT GTT CTT ACC AAT GGA GCA TAT CTT TTG AAC GTG GAT TGT GAT CAT Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val Asp Cys Asp His 15                    100                    105                    110	336
TAC TTT AAT AAC AGT AAG GCT ATT AAA GAA GCT ATG TGT TTC ATG ATG Tyr Phe Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys Phe Met Met 115                    120                    125	384
20 GAC CCG GCT ATT GGA AAG AAG TGC TGC TAT GTC CAG TTC CCT CAA CGT Asp Pro Ala Ile Gly Lys Lys Cys Cys Tyr Val Gln Phe Pro Gln Arg 130                    135                    140	432
25 TTT GAC GGT ATT GAT TTG CAC GAT CGA TAT GCC AAC AGG AAT ATA GTC Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn Arg Asn Ile Val 145                    150                    155                    160	480
30 TTT TTC GAT ATT AAC ATG AAG GGG TTG GAT GGT ATC CAC GGT CCA GTC Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile His Gly Pro Val 165                    170                    175	528
35 TAT GTG GGT ACT GGT TGT TGT TTT AAT AGG CAG GCT CTA TAT GGG TAT Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly Tyr 180                    185                    190	576
GAT CCT GTT TTG ACG GAA GAA GAT TTA GAA CCA AAT ATT ATT GTC AAG Asp Pro Val Leu Thr Glu Glu Asp Leu Glu Pro Asn Ile Ile Val Lys 195                    200                    205	624

- 87 -

AGC TGT TGC GGG TCA AGG AAG AAA GGT AAA AGT AGC AAG AAG TAT AAC			672
Ser Cys Cys Gly Ser Arg Lys Lys Gly Lys Ser Ser Lys Lys Tyr Asn			
210	215	220	
 5 TAC GAA AAG AGG AGA GGC ATC AAC AGA AGT GAC TCC AAT GCT CCA CTT			720
Tyr Glu Lys Arg Arg Gly Ile Asn Arg Ser Asp Ser Asn Ala Pro Leu			
225	230	235	240
 10 TTC AAT ATG GAG GAC ATC GAT GAG GGT TTT GAA CGT TAT GAT GAT GAG			768
Phe Asn Met Glu Asp Ile Asp Glu Gly Phe Glu Gly Tyr Asp Asp Glu			
245	250	255	
 15 AGG TCT ATT CTA ATG TCC CAG AGG AGT GTA GAG AAG CGT TTT GGT CAG			816
Arg Ser Ile Leu Met Ser Gln Arg Ser Val Glu Lys Arg Phe Gly Gln			
260	265	270	
 20 TCG CCG GTA TTT ATT GCG GCA ACC TTC ATG GAA CAA GGC GGC ATT CCA			864
Ser Pro Val Phe Ile Ala Ala Thr Phe Met Glu Gln Gly Gly Ile Pro			
275	280	285	
 25 CCA ACA ACC AAT CCC GCT ACT CTT CTG AAG GAG GCT ATT CAT GTT ATA			912
Pro Thr Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala Ile His Val Ile			
290	295	300	
 30 AGC TGT GGT TAC GAA GAC AAG ACT GAA TGG GGC AAA GAG ATT GGT TGG			960
Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp			
305	310	315	320
 35 ATC TAT GGT TCC GTG ACG GAA GAT ATT CTT ACT GGG TTC AAG ATG CAT			1008
Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His			
325	330	335	
 40 GCC CGG GGT TGG ATA TCG ATC TAC TGC AAT CCT CCA CGC CCT GCG TTC			1056
Ala Arg Gly Trp Ile Ser Ile Tyr Cys Asn Pro Pro Arg Pro Ala Phe			
340	345	350	
 45 AAG GGA TCT GCA CCA ATC AAT CTT TCT GAT CGT TTG AAC CAA GTT CTT			1104
Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu			
355	360	365	

- 88 -

CGA TGG GCT TTG GGA TCT ATC GAG ATT CTT CTT AGC AGA CAT TGT CCT			1152
Arg Trp Ala Leu Gly Ser Ile Glu Ile Leu Leu Ser Arg His Cys Pro			
370	375	380	
 5 ATC TGG TAT GGT TAC CAT GGA AGG TTG AGA CTT TTG GAG AGG ATC GCT			1200
Ile Trp Tyr Gly Tyr His Gly Arg Leu Arg Leu Leu Glu Arg Ile Ala			
385	390	395	400
 10 TAT ATC AAC ACC ATC GTC TAT CCT ATT ACA TCC ATC CCT CTT ATT GCG			1248
Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile Pro Leu Ile Ala			
405	410	415	
 15 TAT TGT ATT CTT CCC GCT TTT TGT CTC ATC ACC GAC AGA TTC ATC ATA			1296
Tyr Cys Ile Leu Pro Ala Phe Cys Leu Ile Thr Asp Arg Phe Ile Ile			
420	425	430	
 20 CCC GAG ATA AGC AAC TAC GCG AGT ATT TGG TTC ATT CTA CTC TTC ATC			1344
Pro Glu Ile Ser Asn Tyr Ala Ser Ile Trp Phe Ile Leu Leu Phe Ile			
435	440	445	
 25 TCA ATT GCT GTG ACT GGA ATC CTG AAA CTG AAA TGG AAC GGT GTG AGC			1392
Ser Ile Ala Val Thr Gly Ile Leu Lys Leu Lys Trp Asn Gly Val Ser			
450	455	460	
 30 TCC ACC CAT CTT TTT GCT GTC TTC CAA GGT CTA CTT AAG GTT CTT GCT			1488
Ser Thr His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala			
485	490	495	
 35 GGT ATC AAC ACC AAC TTC ACC GTT ACA TCT AAA GCC ACA AAC AAA AAT			1536
Gly Ile Asn Thr Asn Phe Thr Val Thr Ser Lys Ala Thr Asn Lys Asn			
500	505	510	
 40 GGG GAT TTT GCA AAA CTC TAC ATC TTC AAA TGG ACA GCT CTT CTC ATT			1584
Gly Asp Phe Ala Lys Leu Tyr Ile Phe Lys Trp Thr Ala Leu Leu Ile			
515	520	525	

- 89 -

CCA CCA ACC ACC GTC CTA CTT GTG AAC CTC ATA GGC ATT GTG GCT GGT Pro Pro Thr Thr Val Leu Leu Val Asn Leu Ile Gly Ile Val Ala Gly	1632
530                   535                   540	
 5 GTC TCT TAT GCT GTA AAC AGT GGC TAC CAG TCG TGG GGT CCG CTT TTC Val Ser Tyr Ala Val Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe	1680
545                   550                   555                   560	
 10 GGG AAG CTC TTC TTC GCC TTA TGG GTT ATT GCC CAT CTC TAC CCT TTC Gly Lys Leu Phe Phe Ala Leu Trp Val Ile Ala His Leu Tyr Pro Phe	1728
15                   565                   570                   575	
 15 TTG AAA GGT CTG TTG GGA AGA CAA AAC CGA ACA CCA ACC ATC GTC ATT Leu Lys Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile	1776
580                   585                   590	
 20 GTC TGG TCT GTT CTC GCC TCC ATC TTC TCG TTG CTT TGG GTC AGG Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg	1824
595                   600                   605	
 25 ATC AAT CCC TTT GTG GAC GCC AAT CCC AAT GCC AAC AAC TTC AAT GGC Ile Asn Pro Phe Val Asp Ala Asn Pro Asn Ala Asn Asn Phe Asn Gly	1872
610                   615                   620	
 25 AAA GGA GGT GTC TTT TAGACCCTAT TTATATACTT GTGTGTGCAT ATATCAAAAA Lys Gly Gly Val Phe	1927
625	
 30 CGCGCAATGG GAATTCCAAA TCATCTAAC CCATCAAACC CCAGTGAACC GGGCAGTTAA	1987
 GGTGATTCCA TGTCAGAT TAGCTTCTC CGAGTAGCCA GAGAAGGTGA ATTGTTCGT	2047
 AACACTATTG TAATGATTTT CCAGTGGGGA AGAAGATGTG GACCCAAATG ATACATAGTC	2107
 35 TACAAAAAGA ATTAGTTATA ACTTTCTTAT ATTTATTTA TTTAAAGCTT GTTAGACTCA	2167
 CACTTATGTA ATGTTGGAAC TTGTTGTCTT AAAAAGGGAT TGGAGTTTTC TTTTATCTA	2227
 40 AGAATCTGAA GTTTATATGC T	2248

- 90 -

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 629 amino acids

5 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala

1 5 10 15

15 Leu Val Ala Lys Ala Gln Lys Ile Pro Gly Glu Gly Trp Thr Met Gln

20 25 30

Asp Gly Thr Pro Trp Pro Gly Asn Asn Thr Arg Asp His Pro Gly Met

35 40 45

20 Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly Asn

50 55 60

Glu Leu Pro Arg Leu Ile Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe

25 65 70 75 80

Gln His His Lys Lys Ala Gly Ala Met Asn Ala Ser Ile Arg Val Ser

85 90 95

30 Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val Asp Cys Asp His

100 105 110

Tyr Phe Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys Phe Met Met

115 120 125

35 Asp Pro Ala Ile Gly Lys Lys Cys Cys Tyr Val Gln Phe Pro Gln Arg

130 135 140

Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn Arg Asn Ile Val

40 145 150 155 160

- 9] -

Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile His Gly Pro Val  
165 170 175

Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly Tyr  
5 180 185 190

Asp Pro Val Leu Thr Glu Glu Asp Leu Glu Pro Asn Ile Ile Val Lys  
195 200 205

10 Ser Cys Cys Gly Ser Arg Lys Lys Gly Lys Ser Ser Lys Lys Tyr Asn  
210 215 220

Tyr Glu Lys Arg Arg Gly Ile Asn Arg Ser Asp Ser Asn Ala Pro Leu  
225 230 235 240

15 Phe Asn Met Glu Asp Ile Asp Glu Gly Phe Glu Gly Tyr Asp Asp Glu  
245 250 255

Arg Ser Ile Leu Met Ser Gln Arg Ser Val Glu Lys Arg Phe Gly Gln  
20 260 265 270

Ser Pro Val Phe Ile Ala Ala Thr Phe Met Glu Gln Gly Gly Ile Pro  
275 280 285

25 Pro Thr Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala Ile His Val Ile  
290 295 300

Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp  
305 310 315 320

30 Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His  
325 330 335

Ala Arg Gly Trp Ile Ser Ile Tyr Cys Asn Pro Pro Arg Pro Ala Phe  
35 340 345 350

Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu  
355 360 365

- 92 -

Arg Trp Ala Leu Gly Ser Ile Glu Ile Leu Leu Ser Arg His Cys Pro  
370 375 380

Ile Trp Tyr Gly Tyr His Gly Arg Leu Arg Leu Leu Glu Arg Ile Ala  
5 385 390 395 400

Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile Pro Leu Ile Ala  
405 410 415

10 Tyr Cys Ile Leu Pro Ala Phe Cys Leu Ile Thr Asp Arg Phe Ile Ile  
420 425 430

Pro Glu Ile Ser Asn Tyr Ala Ser Ile Trp Phe Ile Leu Leu Phe Ile  
435 440 445

15 Ser Ile Ala Val Thr Gly Ile Leu Lys Leu Lys Trp Asn Gly Val Ser  
450 455 460

Ile Glu Asp Trp Trp Arg Asn Asn Gln Phe Trp Val Ile Gly Gly Thr  
20 465 470 475 480

Ser Thr His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala  
485 490 495

25 Gly Ile Asn Thr Asn Phe Thr Val Thr Ser Lys Ala Thr Asn Lys Asn  
500 505 510

Gly Asp Phe Ala Lys Leu Tyr Ile Phe Lys Trp Thr Ala Leu Leu Ile  
515 520 525

30 Pro Pro Thr Thr Val Leu Leu Val Asn Leu Ile Gly Ile Val Ala Gly  
530 535 540

Val Ser Tyr Ala Val Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe  
35 545 550 555 560

Gly Lys Leu Phe Phe Ala Leu Trp Val Ile Ala His Leu Tyr Pro Phe  
565 570 575

- 93 -

Leu Lys Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile  
580 585 590

Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg  
5 595 600 605

Ile Asn Pro Phe Val Asp Ala Asn Pro Asn Ala Asn Asn Phe Asn Gly  
610 615 620

10 Lys Gly Gly Val Phe  
625

- 94 -

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 5           (A) LENGTH: 8411 base pairs  
          (B) TYPE: nucleic acid  
          (C) STRANDEDNESS: single  
          (D) TOPOLOGY: linear

10          (ii) MOLECULE TYPE: DNA (genomic)

          (iii) HYPOTHETICAL: NO

          (iv) ANTI-SENSE: NO

15          (vi) ORIGINAL SOURCE:  
          (A) ORGANISM: *Arabidopsis thaliana*  
          (B) STRAIN: Columbia (wild-type)

20          (vii) IMMEDIATE SOURCE:

          (B) CLONE: 23H12 RSW1 GENE

          (ix) FEATURE:

25          (A) NAME/KEY: exon  
          (B) LOCATION: 2296..2376

          (ix) FEATURE:

30          (A) NAME/KEY: exon  
          (B) LOCATION: 2904..3099

          (ix) FEATURE:

          (A) NAME/KEY: exon  
          (B) LOCATION: 3198..3370

35          (ix) FEATURE:  
          (A) NAME/KEY: exon  
          (B) LOCATION: 3594..3708

40          (ix) FEATURE:

- 95 -

(A) NAME/KEY: exon  
(B) LOCATION: 3824..4013

5 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 4181..4447

10 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 4783..5128

15 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 5207..5344

20 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 5426..5551

25 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 5703..5915

30 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 6022..6286

35 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 6374..6570

(ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 6655..7005

40 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 7088..8032

- 96 -

## (x1) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TTAGAAGAAG CCTGAGCCGG AGTCCTATTTC AATTATCTAG AAGAAGTCTG AGCCGGAGTC	60
5 CCACTCGATT GTCTAGGAGA AGCCTAACGCC GGAGTCCCCT TCGATCACCT AGGAAGAGTG	120
TGACCAGGAG TCCAGTCCGA TCATCTAGGA AGACTGTGAG CAGAAAGTCCG GTTCGTTCAT	180
10 CCAGGAGACG TATCAGCAGG AGTCCAGTCC GATCATCTAG GAAGAGTGTG AGCAGGAGTC	240
CTATTCTGATT GTCCAGAAGA AGTATCAGCA GGAGTCCTAT TCGATTGTCC AGGAGAAAGTA	300
TCAGCAGGAG TCCTGTTAGA GGAAGAAGAA GAATTAGCAG AAGTCCAGTT CCGGCAAGGA	360
15 GAAGGAGTGT GCGGCCTAGA TCTCCTCCTC CTGACCGCAG AAGAAGTTTG TCAAGAAGTG	420
CTTCTCCTAA TGGGCGCATA AGGAGAGGGA GAGGATTAG CCAAAGATTTC TCATACGCC	480
20 GTCGATACAG AACTAGTCCA TCTCCTGATC GATCTCCTTA TCGCTTTAGT GATAGGAGTG	540
ACCGTGACAG GTGAATAGCC CACACATAAT ATAACCCCCC CTTCTGTTA CACACTCTCG	600
TACTGAACCG TCTTTTTAT AACGTCTTCT CTGTAGATT AGAAGTCGCA GAAGGTTCTC	660
25 GCCAAGTCGG TTCAGAACGCC CACTAAGAGG AAGAACACCT CCAAGGTACT TATCCTCTT	720
AGTACATTGT TTCAAGCTGAT TCTTTACATC TAAAAGTTTC ATGAATATGG AACTAAAATT	780
GGTGATCCAA AAGAATTATT CTTGATTTC CAACTCGAAA GTATGTCAG GTATAGAAGA	840
30 AGAACGCCCT CAGTATGCC TGGTCTCTGT TATCGCAACC GGCGGTACAG CCGCAGCCCT	900
ATCCGTAGCC GATCTCCACC TTACAGAAAG AGAAGGTAC CACCGCTAG CCACAGCCTG	960
35 AGTCCATCGA GGTCAAGATC AAGATCAAAG TCATATTCAA AATCTCCCAT TGGGACGGGG	1020
AAAGCAAGAT CAGTGTCAAG ATCACCATCC AAGGCAAGGT CTCCATCGAA GTCGGATTG	1080
40 ACATCCTCCGG ATAATAGCCC AGGTGGAAA AAGGGATTAG TAGCCTATGA TTAATGAATA	1140

- 97 -

ATGATTACCC TTAAGTTAAG TGTTTGTCT TTTTACTGAG AAGAGATGGT AAAGAGAGTA	1200
AGTAGTTTAC TTCTGTAAAA CATAAGCATT GTCTTTGCG TATGTTGTT TGATTATGCT	1260
5 CCAAGATTGT TAAAAATTTC TGTTGATGTT TGCCGACATT TTTTCTTGT TGCCATTGCG	1320
CGACAAATGT TAACCTCCAT TATTCGTTGC GGAGTTGGTT TTGGTCCAAT AATTAAACTT	1380
TCATAAAATT AAGCATAACT AAATGTGACG TTTGTCACCA AACTTTAGAA CAACGACATC	1440
10 GTAATTATT TATTTGGATA ATCAATATAA TTTACGATT CTTCTACAT ATATATCATA	1500
TCACTATACC ACCGTCACTA TCACATCAC TAAATATAAA AATGTTAAAA TGATTTCTA	1560
15 ATGGAATTIT TTTTGTAAA AGTTTATTGA CACAAAAAT GAATTAAAAC TCAGAAATCT	1620
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TCCATTTAAA AATTATTTGC GAATTGGCCC GTAATTCTT AAGCTAACAA TTGAAACCTA	1740
20 ATCAACACTA GTTATTTGA GTCCACCGAC AGGTGATAGC AAATAAAAAA GAACAGGCTG	1800
GTACCAGAGC CAACAACAAAC GTGGCTTCTT CTTTTTTTT TTTAATATAA TCAAACAATC	1860
25 ATACTTGTC CTATCTCTT CTTGCAATAA GATTTGCCA CGTCACATAC TAAGAAGCTG	1920
GCGCGTCTAG TGGGAAGGCC AGAACGGCTC ACTTTAAAAA GTAGAGAGAT GATAACTTGA	1980
GCCGAATAGA GCCGAGCTGA GCTAAAACGG TGGGAGAGGA AGAGGCTACT ACTACCGTCA	2040
30 CCATCTCCGG TAAAATAATG TACTTGTCAT TTAAAAATTA AGAAAAAACCA CATCACTCTG	2100
CGATAAAATA GGCAAAAGCA GATTTGAAGA AGAAGCAGCT TGAGATATCA AATAGAGAGA	2160
35 GAGAGTGACA GAGGAGTGTG TGAACATCCT TTTTGTAGTAG ATTTGGGTTT TCGAGATGCC	2220
GTATTGAATC GGCTACGAAT TTCCCAATT TGAATTGTGTA GAATCTCTCT CTTTCTCTGT	2280
GTGTGGTGG CTGGATGGA GGCCAGTGCC GGCTTGGTTG CTGGATCCTA CGGGAGAAC	2340

- 98 -

GAGCTCGTTC GGATCCGACA TGAATCTGAT GGCGGGGTCT GTTCATCTTC CCTTTTTCCC	2400
ATTTTTTTGT TATTGTTTT CGTTCTTACA ATTTTGATG TGTAGATCTC ATCTAGATTT	2460
5 CTCTGTTCT AAATCTCGTC TCTTTGGAT CCATAATTGG ATCATTGAAA CTCAGATTTC	2520
GCTTCCTTGT ACTGTGTAGT TAGTTAGTGT CAGTTGATCA AGTAAGTGTG TGAAAATGGA	2580
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10 TTTCTTAAAAA TCTCTTATGG AAAGTGTCCG TGGATTTCAAG TTGTTAACT TTTTTAAGCT	2700
AAAATCTTIG ACTCTTAAAG TTTAGCTTTA CTTATTGAGA TTTAGCTCAA CTAGATCTCG	2760
15 TTAGTTCCCG CCATGGGATA CAGACTGTGA CTCGCCTTAA TTCAGATCTG CATTGATTGT	2820
TTTGATTTAG ATCCTTGCTC ATCTCTTCT GTAGTTCTA ATACTCAATG ACTAACAAATG	2880
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20 AGATCTGTGG TGATGATGTT GGACTCGCTG AAACTGGAGA TGTCTTGTC GCGGTGAATG	3000
AAATGTGCCCT CCCTGTGTGT CGGCCTTGCT ATGAGTACGA GAGGAAAGAT CGAACCTCAGT	3060
25 GTTGCCTCA ATGCAAGACT AGATTCAGAC GACACAGGG TCAGTTGTCT TTTTCTTTT	3120
GTTGGCAATT GCTATATATG GATTTTCTCT TTTTGTCTCT TTGCTGTGT GTTGAACAAT	3180
TTTTGGAAT TTTCCAGGGA GTCCTCGTGT TGAAGGAGAT GAAGATGAGG ATGATGTTGA	3240
30 TGATATCGAG AATGAGTTCA ATTACGCCA GGGAGCTAAC AAGGCGAGAC ACCAACGCCA	3300
TGGCGAAGAG TTTCTTCTT CCTCTAGACA TGAATCTCAA CCAATTCTC TTCTCACCCA	3360
35 TGGCCATACG GTAGGGACCT ACATTTCCC TTTAGACTCT AGAGTGATT GTATTACTCA	3420
ATAAAATCCCT AGAGTGGTCA TTTATTACTT ACTATTCAAG TTAATGTTAT ATGTGAACAA	3480
40 ATCTTAAACAG AATTTTTTTC TGATAGTACA TGGTCATCCA AATTAAGAAA TAATAATAGA	3540

- 99 -

TGTTGTTAGT TGTGTCGTT TTCAATAGAT TCATGACCTT TTTCTATACA CAGGTTCTG	3600
GAGAGATTG CACGCCTGAT ACACAATCTG TCGGAACAC ATCAGGTCTT TTGGGTCTT	3660
5 CTGACAGGAA TGCTATTC A TCTCCATATA TTGATCCACG GCAACCTGGT ATTCAATATGT	3720
TTTTCCCTG TGCACGTGGT CTTTGTTAAA TGTGATTCTT ATTCAATTTT ACAACATATA	3780
10 TATTTGTGT ACCGTAAC TG ATAGCTCCCG CTAAAAATTG CAGTCCCTGT AAGAATCGTG	3840
GACCCGTC A AAGACTTGAA CTCTTATGGG CTTGGTAATG TTGACTGGAA AGAAAGAGTT	3900
GAAGGCTGGA AGCTGAAGCA GGAGAAAAAT ATGTTACAGA TGACTGGTAA ATACCAGAA	3960
15 GGGAAAGGAG GAGAAATTGA AGGGACTGGT TCCAATGGCG AAGAACTCCA AATGTAAGTG	4020
GAAATACTAG ACCAATATCT TTATTGTCCA ACTCAAACAG CTCTTGGCCG TGATGCTAAT	4080
AACCACTCTT GGTTTCTTAT TATGTATTGA TAGACATAAT TAAGTATCTG CTTTGTTACA	4140
20 TTTGTTTCTT TCCACTCAAT TATGGTTCTC GTACTACAG GGCTGATGAT ACACGTCTTC	4200
CTATGAGTCG TGTGGTGCGT ATCCCCTCTT CTCGCCTAAC CCCTTATCGG GTTGTGATTAA	4260
25 TTCTCCGCT TATCATCTG TGTGTTCTCT TGCAATATCG TACAACTCAC CCTGTGAAAA	4320
ATGCATATCC TTTGTGGTTG ACCTCGGTTA TCTGTGAGAT CTGGTTGCA TTTCTTGGC	4380
TTCTTGATCA GTTTCCAAA TGGTACCCCA TTAACAGGGA GACTTATCTT GACCGTCTCG	4440
30 CTATAAGTT GGTCTTTAAG TTTATACATC CCCTACTCTC ATCTCTCTT TATGTATTAA	4500
CTTGATATCT TCTATCACAG TTTTCGATAG TTGACTTTT CCCCTGTAA ATTTAATTAA	4560
35 AATTTAGACA ATGGTGCATC TGAATTTGA TTATGATATA TCTTAAGAAG ATTATGATTG	4620
TAAATCTTGA AATTTAGTAG AAAACCATCT GCAATCTACT GACCATGTGA AGTTTCCGAC	4680
40 TAGACTATGA TAGAAGCATG CCAAGTGGAG TGTGTTATTAA GATAGAGCTT AGCTATTATA	4740

- 100 -

	CTGATTTAT ATGTGTTTG ATTTTTGGT TTCTTATTGT AGATATGATC GAGACGGTGA	4800
	ACCATCACAG CTCGTTCTG TTGATGTGTT TGTTAGTACA GTGGACCCAT TGAAAGAGCC	4860
5	TCCCCTTGTT ACAGCAAACA CAGTTCTCTC GATTCTTCT GTGGACTACC CGGTAGATAA	4920
	AGTAGCCTGT TATGTTTCAG ATGATGGTTC AGCTATGCTT ACCTTTGAAT CCCTTTCTGA	4980
	AACCGCTGAG TTTGCAAAGA AATGGGTACC ATTTTGCAAG AAATTCAACA TTGAACCTAG	5040
10	GGCCCCTGAA TTCTATTTG CCCAGAAGAT AGATTACTTG AAGGACAAGA TCCAACCGTC	5100
	TTTTGTTAAA GAGCGACGAG CTATGAAGGT CATTTGAAAA GTCCACCTGC TTCTCATCCA	5160
15	TACGGCAAAG AGATTGACTG ACTTTTTCTT TGGTTGTAT TGACAGAGAG AGTATGAAGA	5220
	GTTTAAAGTG AGGATAAAATG CTCTTGTGTC CAAAGCACAG AAAATCCCTG AAGAAGGCTG	5280
	GACAATGCAG GATGGTACTC CCTGGCCTGG TAACAACACT AGAGATCATC CTGGAATGAT	5340
20	ACAGGTACAG TGTGGCAATC CTTGATTGT GACAGAGAGG ATAACGTAAA GGAAACATGT	5400
	TTACATCGTT TTGTTCAAT TTCAGGTGTT CTTAGGCCAT AGTGGGGTC TGGATACCGA	5460
25	TGGAAATGAG CTGCCAGAC TCATCTATGT TTCTCGTGA AAGCGGCCTG GATTTCACAA	5520
	CCACAAAAAG GCTGGAGCTA TGAATGCATT GGTTTGTAA CTTTCAGAAT CCTATTGTGT	5580
	CCTCTATTTT ATTCTCTTGT TCACTGCCTA AGAAACGTT TCCTTGTGTA GCCGTTGCTT	5640
30	CACATTCTTT TTTTCTAGG CTATGTGTT TCCTCCTAATT TAGTATCTCT TTACTTTGAC	5700
	AGATCCGTGT ATCTGCTGTT CTTACCAATG GAGCATATCT TTTGAACGTG GATTGTGATC	5760
35	ATTACTTTAA TAACAGTAAG GCTATTAAG AAGCTATGTG TTTCATGATG GACCCGGCTA	5820
	TTGGAAAGAA GTGCTGCTAT GTCCAGTTCC CTCAACGTT TGACGGTATT GATTTCACCG	5880
	ATCGATATGC CAACAGGAAT ATAGTCTTTT TCGATGTGAG TATCACTTCC CCATTGTCTT	5940

- 101 -

	TTGTTTCTCT TTTGTCATA TTTGGTTGG ATTTACTCGT TTCTGCTATG GCCTGACTTG	6000
	GATATTTGTT CTCTGGGCA GATTAACATG AAGGGGTTGG ATGGTATCCA GGGTCCAGTA	6060
5	TATGTGGGTA CTGGTTGTTG TTTTAATAGG CAGGCTCTAT ATGGGTATGA TCCTGTTTG	6120
	ACGAAAGAAG ATTTAGAACC AAATATTATT GTCAAGAGCT GTTGCAGGTC AAGGAAGAAA	6180
	GGTAAAAGTA CCAAGAAGTA TAACTACGAA AAGAGGAGAG GCATCAACAG AAGTGACTCC	6240
10	AATGCTCCAC TTTCAATAT GGAGGACATC GATGAGGGTT TTGAAGGTTT GATTGAGCTG	6300
	ATTGTGTAAT AACATCACTT CTTTATGTA TGATTATGT GATGGTGAAA TCTTACAATC	6360
15	CTTGTATG CAGGTTATGA TGATGAGAGG TCTATTCTAA TGTCCCAGAG GAGTGTAGAG	6420
	AAGCGTTTG GTCAGTCGCC GGTATTTATT GCGGCAACCT TCATGGAACA AGCGGCATT	6480
	CCACCAACAA CCAATCCCGC TACTCTCTG AAGGAGGCTA TTCATGTTAT AAGCTGTGGT	6540
20	TACGAAGACA AGACTGAATG GGGCAAAGAG GTCAGTTTC AAATGCAGCT ACAGAATCTT	6600
	CTTATGTTCT CTTCTTACC TGTTGATGA CATCTTATTT GGCACTTTG TTAGATTGGT	6660
25	TGGATCTATG GTTCCGTGAC GGAAGATATT CTTACTGGGT TCAAGATGCA TGCCCGGGGT	6720
	TGGATATCGA TCTACTGCAA TCCTCCACGC CCTGCCTCA AGGGATCTGC ACCAATCAAT	6780
	CTTCTGATC GTTGAAACCA AGTTCTCGA TGGGCTTTGG GATCTATCGA GATTCTCTT	6840
30	AGCAGACATT GTCCTATCTG GTATGGTTAC CATGGAAGGT TGAGACTTTT GGAGAGGATC	6900
	GCTTATATCA ACACCATCGT CTATCCATT ACATCCATCC CTCTTATTCG GTATTGTATT	6960
35	CTTCCCGCTT TTTGTCTCAT CACCGACAGA TTCATCATAAC CCGAGGTTTG TAAAAGTGAC	7020
	CACACTGCTA TTTACTATTT GAATCCCATT TTGTGAATGC ATTTTTTGT CATCATCATT	7080
	GTTGCAGATA AGCAACTACG CGAGTATTG GTTCATTCTA CTCTTCATCT CAATTGCTGT	7140
40		

- 102 -

10	GACTGGAATC CTGGAGCTGA GATGGACCGG TGTGAGCATT GAGGATTGGT GGAGGAACGA	7200
	GCAGTTCTGG GTCATTGGTG GCACATCCGC CCATCTTTT GCTGTCTTCC AAGGTCTACT	7260
5	TAAGGTTCTT GCTGGTATCG ACACCAACTT CACCGTTACA TCTAAAGCCA CAGACGAAGA	7320
	TGGGGATTTT GCAGAACTCT ACATCTTCAA ATGGACAGCT CTTCTCATTC CACCAACCAC	7380
	CGTCCTACTT GTGAACCTCA TAGGCATTGT GGCTGGTGT CTCATGCTG TAAACAGTGG	7440
10	CTACCAAGTCG TGGGGTCCGC TTTTCCGGAA GCTCTTCTTC GCCTTATGGG TTATTGCCA	7500
	TCTCTACCCCT TTCTTGAAAG GTCTGTTGGG AAGACAAAAC CGAACACCAA CCATCGTCAT	7560
15	TGTCTGGTCT GTTCTTCTCG CCTCCATCTT CTCGTTGCTT TGGGTCAAGGA TCAATCCCTT	7620
	TGTGGACGCC AATCCAATG CCAACAACCTT CAATGGCAAA GGAGGTGTCT TTAGACCCCT	7680
	ATTTATATAAC TTGTGTGTGC ATATATCAA AACGCGCAAT GGGATTCCA AATCATCTAA	7740
20	ACCCATCAAAC CCCCAAGTGAA CCGGGCAGTT AAGGTGATTC CATGTCCAAG ATTAGCTTTC	7800
	TCCGAGTAGC CAGAGAAGGT GAAATTGTTCA GTAACACTAT TGTAATGATT TTCCAGTGGG	7860
25	GAAGAAGATG TGGACCCAAA TGATACATAG TCTACAAAAA GAATTGTTA TTCTTTCTTA	7920
	TATTTATTTT ATTTAAAGCT TGTTAGACTC ACACCTATGT AATGTGGAA CTTGTGTGCC	7980
	TAAGGAAAGGA TTGGAGTTTT CTTTTTATCT AAGAATCTGA AGTTTATATG CTAAGCTTTT	8040
30	CACTTTACTA CAAAAAGTTT ATGGATATGA TGGTGTACGT CAATTGTTGG TGCAAGTGT	8100
	GATGTCTTCG GGTGAACCTCG CCCTCTTGTGTT TTGTCTCACCC CATCAGTACA AATAGAATGA	8160
35	CATTTATTTT TTGAACTTT TAACGAAATC TTTGTCATTA TGGGACTTGA TCAGTAAAGT	8220
	TACATATTTG AAGAGATATT GTGTAAACTC TTATTTGAAT CAGAACAGA TCAATCAAAA	8280
	ATTGAAAACG TAAAGTTCAA ACAAAAAGGT AGAGTGAATC TTTTAATCCC CCCTCAATAC	8340

- 103 -

TAATTTGTGA AATCTCAAGT GGTGTAAAAT GAACCCAATT AGTATCCACA ATGTGTTTCT 8400

CTGATCAATC C 8411

5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 5009 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20 (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Arabidopsis thaliana  
(B) STRAIN: Columbia

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: 12C4

(ix) FEATURE:

30 (A) NAME/KEY: exon  
(B) LOCATION: 863..943

(ix) FEATURE:

(A) NAME/KEY: exon  
(B) LOCATION: 1454..1840

35

(ix) FEATURE:

(A) NAME/KEY: exon  
(B) LOCATION: 1923..2025

40

(ix) FEATURE:

- 104 -

(A) NAME/KEY: exon  
(B) LOCATION: 2122..2311

(ix) FEATURE:

5 (A) NAME/KEY: exon  
(B) LOCATION: 2421..2687

(ix) FEATURE:

10 (A) NAME/KEY: exon  
(B) LOCATION: 2776..3121

(ix) FEATURE:

15 (A) NAME/KEY: exon  
(B) LOCATION: 3220..3357

15 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 3507..3623

20 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 3723..3935

25 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 4027..4297

30 (ix) FEATURE:  
(A) NAME/KEY: exon  
(B) LOCATION: 4380..4576

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

35 AAGGAATAAT AAGATAGGGG TTTAATGGGA GACAATCAAT CTTCAGGGGT TTTCTGGAAN 60

AACGGCGGGG TAAAAAACAA GACATCAATC GGACCCGATC ACGAGGACCC GGATCCGNAT 120

CGATAAACAG NGTAGCTTTC AATACCCCAT TTTCCCAGAA ACACCTCTCA AAAATTTTT 180

- 105 -

CAAGAACTNG TATAAATATC TCAGTTTGT TCACGCAGGT CTTINNTATT TTGGNAANTC	240
TNTNTTCATN GTTCACCAAC TCCCTCTTGA AGGTGGGACA GAGTCCAGCT CCACCACCAC	300
5 CATAGCCATC GCGTCGTTT CTCCGGGACC CACTTATTC GTGACGTTTC TCTCTTGTA	360
TATACATACA ATTGTTTCA GTCTCAATTG GCTGCCACA TTTAACACA ACTCTATCTC	420
AGGGGTGGTG TCTGAATCTC GTCTCTCTCA TTCCTATTA TCCCAATCTA ATCTATCACA	480
10 AACCCCTCCA CATTGCTTTT GTCAGTCTGT AAAATTCTCT TTGAATCAGT GAATCACTCA	540
CTTAAATCCA AAACAGTTT TTTTCTTTC TTTCTTATT TGCTTGTGT GGAATCAATA	600
15 GCTGTCTCCG GGAAAATTCG TTTTTTTCT CCTTCGGGAT CTCTTTTTT TTTTTTTGG	660
TTTATTTAA TAATTATCCC CGAGCCAACA TTTATTGTCG ATTGGTTTA TTTCGTCTCC	720
20 TTCGTCTTCC ACTCTTACTA GTGCATGCTC TGAATCTGA TGTAATGGGA GTCAACAGT	780
CTGGATCCAT TATCCTAGCC GGGTCGGGTC AAGGTTTTG AGTAAGAGAG ACAATTGTT	840
TTGATTGGT GTAGAAGACA TCATGAATAC TGGTGGTCGG CTCATTGCTG GCTCTCACAA	900
25 CAGAAACGAA TTCGTTCTCA TTAACGCCGA TGAGAGTGCC AGAGTAAGAA TAACTTTGT	960
ANGAATTGT GACGGAAAAA AGTTAATTG TTTCTTTTC TTGGGGATCT AGATTATGAG	1020
AATCTAGATG GAATATTTTG ATCTGAAATT GGAAGTTCT AGGGAGTAAT GCGCAACCC	1080
30 ACATGTTCTG TTTTTCTTT TTTCTTTCT TCAAGTAGTG TTGCATGATT CATACTGTC	1140
GGCAGAGATG TCCGAGAAC CGAATTCAAT GTGTAGCAG TAGCAATAAG TTCAAAGAAA	1200
35 GTCCATTTT TTATATTACT AATTCTGTTG TTGGTTTATT TGAGCTGGTC TTTATTGCAT	1260
TTCACCTGGA TTCAGATACT AATAACTGTC TCAATTATGT AAAAATGACA ACTTTATGAA	1320
40 ATTCAGTTTC ACAATTATGT AATTCTATAAT CGATGAATGT TTTCTTGAG TCTTTATCAT	1380

- 106 -

CTTTAGGATT TGATTAAGAT GCAATTTGAT GAAAATACTA AAAAGACTCA TGTGTTCTCA	1440
TTTCTCTATG TAGATACGAT CAGTACAAGA ACTGAGTGGG CAAACATGTC AAATCTGTGG	1500
5 AGATGAAATC GAATTAACGG TTAGCAGTGA GCTCTTGTT GCTTGCACCG AATGCGCATT	1560
CCCGGTTTGT AGACCATGCT ATGACTATGA ACGTAGAGAA CGAAATCAAG CTTGTCCTCA	1620
10 GTGCAAAACT CGATACAAAA GGATTAAGG TAGTCCACGG GTTGATGGAG ATGATGAAGA	1680
AGAAGAAGAC ATTGATGATC TTGAGTATGA GTTGTGATCAT GGGATGGACC CTGAACATGC	1740
CGCTGAAGCC GCACTCTCTT CACGCCCTAA CACCGGTCGT GGTGGATTGG ATTCAAGCTCC	1800
15 ACCTGGCTCT CAGATTCCTC TTTTGACTTA TTGTGATGAA GTGAGGAATC CAAATTGTTT	1860
GTTTCTCTG ACAATGTGT TGCTTAGATG ATTCTTTTC TTATTAGTCT ATGTGTTTC	1920
20 AGGATGCTGA TATGTATTCT GATCGTCATG CTCTTATCGT GCCTCCTTCA ACAGGGATATG	1980
GGAATCGCGT CTATCCTGCA CCGTTTACAG ATTCTTCTGC ACCTCGTATG TGTTTACTTT	2040
TATGATTCCCT ACAATTTTC TTCTTATATG ATTTGGTCAC CTTCTAATGA GTTATGAAAT	2100
25 GGTTTTGTTT GTGTTTTCA GCACAGGCGA GATCAATGGT TCCTCAGAAA GATATTGCGG	2160
AATATGGTTA TCGAAGTGTGTT GCTTGGAAAGG ACCGTATGGA AGTTTGGAAAG AGACGGACAAG	2220
30 GCGAAAAGCT TCAAGTCATT AAGCATGAAG GAGGAAACAA TGGTCGAGGT TCCAATGATG	2280
ACGACGAACt AGATGATCCT GACATGCCTA TGTAAGTTGT TAAAATCTAA CAAAAGTTCA	2340
GATGAAATGA TGCTCTGAAA TTTTGTGTTA AATGGNTTIG TTTTCTTATT GTTGTAA	2400
35 CATTTCCTG GCTAATTCAAG GATGGATGAA GGAAGACAAC CTCTCTCAAG AAAGCTACCT	2460
ATTCGTTCAA GCAGAATAAA TCCTTACAGG ATGTTAACCC TGTGTCGCCT CGCGATTCTT	2520
40 GGTCTTTCTT TTCATTATAG AATTCTCCAT CCAGTCATG ATGCATATGG ATTATGGTTA	2580

- 107 -

ACGTCAGTTA TATGCGAGAT ATGGTTGCA GTGTCTTGGAA TTCTTGATCA ATTCCCCAAA	2640
TGGTATCCTA TAGAACGTGA AACATACCTC GATAGACTCT CTCTCAGGTA ACATAAACCC	2700
5 TGAAAAGTTC TTGTCGCAA ATATTCATTG TTTACATTCC CAAAAATTGT TGAAACTCTA	2760
TTTTCTTAC ATAAGGTACG AGAAGGAAGG AAAACCGTCA GGATTAGCAC CTGTTGATGT	2820
TTTGTTAGT ACAGTGGATC CGTTGAAAGA GCCACCCCTG ATTACAGCAA ACACAGTTCT	2880
10 TTCCATTCTA GCAGTTGATT ATCCTGTGGA TAAGGTTGCG TGTTATGTAT CAAACAATGG	2940
TGCAGCTATG CTTACATTG AAGCTCTCTC TGATACAGCT GAGTTTGCTA GAAAATGGGT	3000
15 TCCTTTTGT AAGAAGTTTA ATATCGAGCC ACGAGCTCCT GAGTGGTATT TTTCTCAGAA	3060
GATGGATTAC CTGAAGAACAA AAGTTCATCC TGCTTTGTC AGGGAACGTC GTGCTATGAA	3120
GGTTTTCTT GCTGCTTTT CTCTTTCTGA GTATATCCTA TCATAAAAGT GTGTTTCAA	3180
20 GAATCTGATT TACGTTTTT GCTTGTGTT GTGTTGAGA GAGATTATGA GGAGTTTAAA	3240
GTGAAGATAA ATGCACTGGT TGCTACTGCA CAGAAAGTGC CTGAGGAAGG TTGGACTATG	3300
25 CAAGATGGAA CTCCCTGGCC TGGAAACAAAC GTCCGTGACC ATCCTGGAAT GATTCAAGGTA	3360
ATGATGAGTT TGATTGAATA GGCAAAAAAA AAGCGGTTTT TGTCTCTTC ACTTTGTTTC	3420
CCTGGATCTG TAAATTGGA ATGAGCACTC TACTTCTCAA TATATCTTCA GACCGAAGCC	3480
30 TTTTAAGAG ATTTTGAAA TGACAGGTGT TCTTGGGTCA TAGTGGAGTT CGTGATACGG	3540
ATGGTAATGA GTTACCAACGT CTAGTGTATG TTTCTCGTGA GAAGCGGCCT GGATTTGATC	3600
35 ACCACAAGAA AGCTGGAGCT ATGAATTCCCT TGGTAAGTAT AATGTGTTTC TTTATTTATG	3660
AATCTCTCTT TTGGAGCCC TGACTTCTCA TAAACTAAAA CTCATCTTAC TTCTTCTTGA	3720
40 AGATCCGAGT CTCTGCTGTT CTATCAAACG CTCCCTACCT TCTTAATGTC GATTGTGATC	3780

- 108 -

	ACTACATCAA CAACAGCAAA GCAATTAGAG AATCTATGTG TTTCATGATG GACCCGCAAT	3840
	CGGGAAAGAA AGTTTGTAT GTTCAGTTTC CGCAGAGATT TGATGGGATT GATAGACATG	3900
5	ATAGATACTC AAACCGTAAC GTTGTGTTCT TTGATGTATG TGTCCTTATC TCTTTGCTT	3960
	TGTTTCTGTT TATGTTTAG TGCTTTCTCT CTTTCTCAT TTGATATTGT TTTGGTGTGG	4020
	AAACAGATTA ACATGAAAGG TCTTGATGGG ATACAAGGAC CGATATATGT CGGGACAGGT	4080
10	TGTGTGTTA GAAAACAGGC TCTTATGGT TTTGATGCAC CAAAGAAGAA GAAACCACCA	4140
	GGCAAAACCT GTAATGTTG GCCTAAATGG TGTGTGTTGT GTTGTGGTT GAGAAAGAAG	4200
15	AGTAAAACGA AAGCCAAAGA TAAGAAAATC AACACTAAAG AGACTTCAAA GCAGATTCA	4260
	GCGCTAGAGA ATGTCGACGA AGGTGTTATC GTCCCAGGTA AAAAAAGAAG GAAAAAAAAA	4320
	ACATTTCTTA TTTGGTTCT GTCTGTTGA AAGTCTAAGT AGATCCTTT GATTGTTAGT	4380
20	GTCAAATGTT GAGAAGAGAT CTGAAGCAAC ACAATTGAAA TTGGAGAAGA AGTTTGGACA	4440
	ATCTCCGGTT TTCGTTGCCT CTGCTGTTCT ACAGAACGGT GGAGTTCCCC GTAACGCAAG	4500
25	CCCCGCATGT TTGTTAAGAG AAGCCATTCA AGTTATTAGC TGCGGGTACG AAGATAAAAC	4560
	CGAATGGGGA AAAGAGGTAG AAAACATTAC AAAGTTTTTC AACTCTGAA AACTAGAAAA	4620
	GTTCTTGTGA TCTCATTCTT GCTGATAATC ACACGCAGAT CGGGTGGATT TATGGATCGG	4680
30	TGACTGAAGA TATCCTGACG GGTTTCAAGA TGCATTGCCA TGGATGGAGA TCTGTGTACT	4740
	GTATGCCTAA GCGTGCAGCT TTTAAAGGAT CTGCTCTAT TAACTGTCA GATCGCTTC	4800
35	ATCAAGTTCT ACGTTGGCT CTTGGCTCTG TAGAGATTTT CTTGAGCAGA CATTGTCCGA	4860
	TATGGTATGG TTATGGTGGT GGTTAAAAT GGTTGGAGAG ATTCTCTTAC ATCAACTCTG	4920
	TCGTCTATCC TTGGACTTCA CTTCCATTGA TCGTCTATTG TTCTCTCCCC GCGGTTTGT	4980

- 109 -

TACTCACAGG AAAATTCAAC GTCCCTGAG

5009

## 5 (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3603 base pairs  
(B) TYPE: nucleic acid  
10 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Arabidopsis thaliana  
(B) STRAIN: Columbia

20

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: RSW1 cDNA

25

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1..3243

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG GAG GCC AGT GCC GGC TTG GTT GCT GGA TCC TAC CGG AGA AAC GAG

48

Met Glu Ala Ser Ala Gly Leu Val Ala Gly Ser Tyr Arg Arg Asn Glu

1

5

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CTC GTT CGG ATC CGA CAT GAA TCT GAT GGC GGG ACC AAA CCT TTG AAG  
Leu Val Arg Ile Arg His Glu Ser Asp Gly Gly Thr Lys Pro Leu Lys

96

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- 110 -

AAT ATG AAT GGC CAG ATA TGT CAG ATC TGT GGT GAT GAT GTT GGA CTC	144		
Asn Met Asn Gly Gln Ile Cys Gln Ile Cys Gly Asp Asp Val Gly Leu			
35	40	45	
 5 GCT GAA ACT GGA GAT GTC TTT GTC GCG TGT AAT GAA TGT GCC TTC CCT	192		
Ala Glu Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala Phe Pro			
50	55	60	
 10 GTG TGT CGG CCT TGC TAT GAG TAC GAG AGG AAA GAT GGA ACT CAG TGT	240		
Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln Cys			
65	70	75	80
 15 TGC CCT CAA TGC AAG ACT AGA TTC AGA CGA CAC AGG GGG AGT CCT CGT	288		
Cys Pro Gln Cys Lys Thr Arg Phe Arg Arg His Arg Gly Ser Pro Arg			
85	90	95	
 20 GTT GAA GGA GAT GAA GAT GAG GAT GAT GTT GAT GAT ATC GAG AAT GAG	336		
Val Glu Gly Asp Glu Asp Glu Asp Asp Val Asp Asp Ile Glu Asn Glu			
100	105	110	
 25 TTC AAT TAC GCC CAG GGA GCT AAC AAG GCG AGA CAC CAA CGC CAT GGC	384		
Phe Asn Tyr Ala Gln Gly Ala Asn Lys Ala Arg His Gln Arg His Gly			
115	120	125	
 30 GAA GAG TTT TCT TCT TCC TCT AGA CAT GAA TCT CAA CCA ATT CCT CTT	432		
Glu Glu Phe Ser Ser Ser Arg His Glu Ser Gln Pro Ile Pro Leu			
130	135	140	
 35 CTC ACC CAT GGC CAT ACG GTT TCT GGA GAG ATT CGC ACG CCT GAT ACA	480		
Leu Thr His Gly His Thr Val Ser Gly Glu Ile Arg Thr Pro Asp Thr			
145	150	155	160
 40 CAA TCT GTG CGA ACT ACA TCA GGT CCT TTG GGT CCT TCT GAC AGG AAT	528		
Gln Ser Val Arg Thr Thr Ser Gly Pro Leu Gly Pro Ser Asp Arg Asn			
165	170	175	
 45 GCT ATT TCA TCT CCA TAT ATT GAT CCA CGG CAA CCT GTC CCT GTA AGA	576		
Ala Ile Ser Ser Pro Tyr Ile Asp Pro Arg Gln Pro Val Pro Val Arg			
180	185	190	

- 111 -

ATC GTG GAC CCG TCA AAA GAC TTG AAC TCT TAT GGG CTT GGT AAT GTT Ile Val Asp Pro Ser Lys Asp Leu Asn Ser Tyr Gly Leu Gly Asn Val 195                    200                    205	624
 5 GAC TGG AAA GAA AGA GTT GAA GGC TGG AAG CTG AAG CAG GAG AAA AAT Asp Trp Lys Glu Arg Val Glu Gly Trp Lys Leu Lys Gln Glu Lys Asn 210                    215                    220	672
 ATG TTA CAG ATG ACT GGT AAA TAC CAT GAA GGG AAA GGA GGA GAA ATT 10 Met Leu Gln Met Thr Gly Lys Tyr His Glu Gly Lys Gly Glu Ile 225                    230                    235                    240	720
 GAA GGG ACT GGT TCC AAT GGC GAA GAA CTC CAA ATG GCT GAT GAT ACA Glu Gly Thr Gly Ser Asn Gly Glu Glu Leu Gln Met Ala Asp Asp Thr 15                    245                    250                    255	768
 CGT CTT CCT ATG AGT CGT GTG GTG CCT ATC CCA TCT TCT CGC CTA ACC Arg Leu Pro Met Ser Arg Val Val Pro Ile Pro Ser Ser Arg Leu Thr 260                    265                    270	816
 20 CCT TAT CGG GTT GTG ATT ATT CTC CGG CTT ATC ATC TTG TGT TTC TTC Pro Tyr Arg Val Val Ile Ile Leu Arg Leu Ile Ile Leu Cys Phe Phe 275                    280                    285	864
 25 TTG CAA TAT CGT ACA ACT CAC CCT GTG AAA AAT GCA TAT CCT TTG TGG Leu Gln Tyr Arg Thr Thr His Pro Val Lys Asn Ala Tyr Pro Leu Trp 290                    295                    300	912
 TTG ACC TCG GTT ATC TGT GAG ATC TGG TTT GCA TTT TCT TGG CTT CTT 30 Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Phe Ser Trp Leu Leu 305                    310                    315                    320	960
 GAT CAG TTT CCC AAA TGG TAC CCC ATT AAC AGG GAG ACT TAT CTT GAC Asp Gln Phe Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp 35                    325                    330                    335	1008
 CGT CTC GCT ATA AGA TAT GAT CGA GAC GGT GAA CCA TCA CAG CTC GTT Arg Leu Ala Ile Arg Tyr Asp Arg Asp Gly Glu Pro Ser Gln Leu Val 340                    345                    350	1056

- 112 -

CCT GTT GAT GTG TTT GTT AGT ACA GTG GAC CCA TTG AAA GAG CCT CCC			1104
Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro			
355	360	365	
 5 CTT GTT ACA GCA AAC ACA GTT CTC TCG ATT CTT TCT GTG GAC TAC CCG			1152
Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro			
370	375	380	
 10 GTA GAT AAA GTA GCC TGT TAT GTT TCA GAT GAT GGT TCA GCT ATG CTT			1200
Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ser Ala Met Leu			
385	390	395	400
 15 ACC TTT GAA TCC CTT TCT GAA ACC GCT GAG TTT GCA AAG AAA TGG GTA			1248
Thr Phe Glu Ser Leu Ser Glu Thr Ala Glu Phe Ala Lys Lys Trp Val			
405	410	415	
 20 CCA TTT TGC AAG AAA TTC AAC ATT GAA CCT AGG GCC CCT GAA TTC TAT			1296
Pro Phe Cys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr			
420	425	430	
 25 TTT GCC CAG AAG ATA GAT TAC TTG AAG GAC AAG ATC CAA CCG TCT TTT			1344
Phe Ala Gln Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser Phe			
435	440	445	
 30 GTT AAA GAG CGA CGA GCT ATG AAG AGA GAG TAT GAA GAG TTT AAA GTG			1392
Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val			
450	455	460	
 35 AGG ATA AAT GCT CTT GTT GCC AAA GCA CAG AAA ATC CCT GAA GAA GGC			1440
Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Ile Pro Glu Glu Gly			
465	470	475	480
 40 TGG ACA ATG CAG GAT GGT ACT CCC TGG CCT GGT AAC AAC ACT AGA GAT			1488
Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Thr Arg Asp			
485	490	495	
 45 CAT CCT GGA ATG ATA CAG GTG TTC TTA GGC CAT AGT GGG GGT CTG GAT			1536
His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp			
500	505	510	

- 113 -

ACC GAT GGA AAT GAG CTG CCT AGA CTC ATC TAT GTT TCT CGT GAA AAG			1584
Thr Asp Gly Asn Glu Leu Pro Arg Leu Ile Tyr Val Ser Arg Glu Lys			
515	520	525	
 5 CGG CCT GGA TTT CAA CAC CAC AAA AAG GCT GGA GCT ATG AAT GCA TTG			1632
Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu			
530	535	540	
 ATC CGT GTA TCT GCT GTT CTT ACC AAT GGA GCA TAT CTT TTG AAC GTG			1680
10 Ile Arg Val Ser Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val			
545	550	555	560
 GAT TGT GAT CAT TAC TTT AAT AAC AGT AAG GCT ATT AAA GAA GCT ATG			1728
Asp Cys Asp His Tyr Phe Asn Asn Ser Lys Ala Ile Lys Glu Ala Met			
15	565	570	575
 TGT TTC ATG ATG GAC CCG GCT ATT GGA AAG AAG TGC TGC TAT GTC CAG			1776
Cys Phe Met Met Asp Pro Ala Ile Gly Lys Lys Cys Cys Tyr Val Gln			
580	585	590	
20 TTC CCT CAA CGT TTT GAC GGT ATT GAT TTG CAC GAT CGA TAT GCC AAC			1824
Phe Pro Gln Arg Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn			
595	600	605	
 25 AGG AAT ATA GTC TTT TTC GAT ATT AAC ATG AAG GGG TTG GAT GGT ATC			1872
Arg Asn Ile Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile			
610	615	620	
 CAG GGT CCA GTA TAT GTG GGT ACT GGT TGT TGT TTT AAT AGG CAG GCT			1920
30 Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala			
625	630	635	640
 CTA TAT GGG TAT GAT CCT GTT TTG ACG GAA GAA GAT TTA GAA CCA AAT			1968
Leu Tyr Gly Tyr Asp Pro Val Leu Thr Glu Glu Asp Leu Glu Pro Asn			
35	645	650	655
 ATT ATT GTC AAG AGC TGT TGC GGG TCA AGG AAG AAA GGT AAA AGT AGC			2016
Ile Ile Val Lys Ser Cys Cys Gly Ser Arg Lys Lys Gly Lys Ser Ser			
660	665	670	

- 114 -

- 115 -

AGA CAT TGT CCT ATC TGG TAT GGT TAC CAT GGA AGG TTG AGA CTT TTG	2544		
Arg His Cys Pro Ile Trp Tyr Gly Tyr His Gly Arg Leu Arg Leu Leu			
835	840	845	
 5 GAG AGG ATC GCT TAT ATC AAC ACC ATC GTC TAT CCT ATT ACA TCC ATC	2592		
Glu Arg Ile Ala Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile			
850	855	860	
 CCT CTT ATT GCG TAT TGT ATT CTT CCC GCT TTT TGT CTC ATC ACC GAC	2640		
10 Pro Leu Ile Ala Tyr Cys Ile Leu Pro Ala Phe Cys Leu Ile Thr Asp			
865	870	875	880
 AGA TTC ATC ATA CCC GAG ATA AGC AAC TAC GCG AGT ATT TGG TTC ATT	2688		
Arg Phe Ile Ile Pro Glu Ile Ser Asn Tyr Ala Ser Ile Trp Phe Ile			
15	885	890	895
 CTA CTC TTC ATC TCA ATT GCT GTG ACT GGA ATC CTG GAG CTG AGA TGG	2736		
Leu Leu Phe Ile Ser Ile Ala Val Thr Gly Ile Leu Glu Leu Arg Trp			
900	905	910	
20			
AGC GGT GTG AGC ATT GAG GAT TGG TGG AGG AAC GAG CAG TTC TGG GTC	2784		
Ser Gly Val Ser Ile Glu Asp Trp Trp Arg Asn Glu Gln Phe Trp Val			
915	920	925	
 25 ATT GGT GGC ACA TCC GCC CAT CTT TTT GCT GTC TTC CAA GGT CTA CTT	2832		
Ile Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu			
930	935	940	
 AAG GTT CTT GCT GGT ATC GAC ACC AAC TTC ACC GTT ACA TCT AAA GCC	2880		
30 Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala			
945	950	955	960
 ACA GAC GAA GAT GGG GAT TTT GCA GAA CTC TAC ATC TTC AAA TGG ACA	2928		
Thr Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Ile Phe Lys Trp Thr			
35	965	970	975
 GCT CTT CTC ATT CCA CCA ACC ACC GTC CTA CTT GTG AAC CTC ATA GGC	2976		
Ala Leu Leu Ile Pro Pro Thr Thr Val Leu Leu Val Asn Leu Ile Gly			
980	985	990	

- 116 -

ATT GTG GCT GGT GTC TCT TAT GCT GTA AAC AGT GGC TAC CAG TCG TGG Ile Val Ala Gly Val Ser Tyr Ala Val Asn Ser Gly Tyr Gln Ser Trp 995                    1000                    1005	3024
5 GGT CCG CTT TTC GGG AAG CTC TTC TCC GCC TTA TGG GTT ATT GCC CAT Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Leu Trp Val Ile Ala His 1010                    1015                    1020	3072
10 CTC TAC CCT TTC TTG AAA GGT CTG TTG GGA AGA CAA AAC CGA ACA CCA Leu Tyr Pro Phe Leu Lys Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro 1025                    1030                    1035                    1040	3120
15 ACC ATC GTC ATT GTC TGG TCT GTT CTT CTC GCC TCC ATC TTC TCG TTG Thr Ile Val Ile Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu 1045                    1050                    1055	3168
20 CTT TGG GTC AGG ATC AAT CCC TTT GTG GAC GCC AAT CCC AAT GCC AAC Leu Trp Val Arg Ile Asn Pro Phe Val Asp Ala Asn Pro Asn Ala Asn 1060                    1065                    1070	3216
AAC TTC AAT GGC AAA GGA GGT GTC TTT TAGACCCTAT TTATATACTT Asn Phe Asn Gly Lys Gly Val Phe 1075                    1080	3263
25 GTGTGTGCAT ATATCAAAAA CGCGCAATGG GAATTCCAAA TCATCTAAC CCATCAAACC	3323
CCAGTGAACC GGGCAGTTAA GGTGATTCCA TGTCCAAGAT TAGCTTCTC CGAGTAGCCA	3383
30 GAGAAGGTGA AATTGTTCGT AACACTATTG TAATGATTTT CCAGTGGGA AGAAGATGTG GACCCAAATG ATACATAGTC TACAAAAAGA ATTTGTTATT CTTTCTTATA TTTATTTTAT	3443                    3503
TTAAAGCTTG TTAGACTCAC ACTTATGTAA TGTTGGAAC TGTGTCCTA AAAAGGGATT	3563
35 GGAGTTTCTT TTTTATCTAA GAATCTGAAG TTTATATGCT	3603

(2) INFORMATION FOR SEQ ID NO:6:

40                    (i) SEQUENCE CHARACTERISTICS:

- 117 -

- (A) LENGTH: 1081 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Ala Ser Ala Gly Leu Val Ala Gly Ser Tyr Arg Arg Asn Glu  
10 1 5 10 15

Leu Val Arg Ile Arg His Glu Ser Asp Gly Gly Thr Lys Pro Leu Lys  
20 25 30

15 Asn Met Asn Gly Gln Ile Cys Gln Ile Cys Gly Asp Asp Val Gly Leu  
35 40 45

Ala Glu Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala Phe Pro  
50 55 60

20 Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln Cys  
65 70 75 80

Cys Pro Gln Cys Lys Thr Arg Phe Arg Arg His Arg Gly Ser Pro Arg  
25 85 90 95

Val Glu Gly Asp Glu Asp Asp Val Asp Asp Ile Glu Asn Glu  
100 105 110

30 Phe Asn Tyr Ala Gln Gly Ala Asn Lys Ala Arg His Gln Arg His Gly  
115 120 125

Glu Glu Phe Ser Ser Ser Arg His Glu Ser Gln Pro Ile Pro Leu  
130 135 140

35 Leu Thr His Gly His Thr Val Ser Gly Glu Ile Arg Thr Pro Asp Thr  
145 150 155 160

Gln Ser Val Arg Thr Thr Ser Gly Pro Leu Gly Pro Ser Asp Arg Asn  
40 165 170 175

- 118 -

Ala Ile Ser Ser Pro Tyr Ile Asp Pro Arg Gln Pro Val Pro Val Arg  
180 185 190

Ile Val Asp Pro Ser Lys Asp Leu Asn Ser Tyr Gly Leu Gly Asn Val  
5 195 200 205

Asp Trp Lys Glu Arg Val Glu Gly Trp Lys Leu Lys Gln Glu Lys Asn  
210 215 220

10 Met Leu Gln Met Thr Gly Lys Tyr His Glu Gly Lys Gly Glu Ile  
225 230 235 240

Glu Gly Thr Gly Ser Asn Gly Glu Glu Leu Gln Met Ala Asp Asp Thr  
245 250 255

15 Arg Leu Pro Met Ser Arg Val Val Pro Ile Pro Ser Ser Arg Leu Thr  
260 265 270

Pro Tyr Arg Val Val Ile Ile Leu Arg Leu Ile Ile Leu Cys Phe Phe  
20 275 280 285

Leu Gln Tyr Arg Thr Thr His Pro Val Lys Asn Ala Tyr Pro Leu Trp  
290 295 300

25 Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Phe Ser Trp Leu Leu  
305 310 315 320

Asp Gln Phe Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp  
325 330 335

30 Arg Leu Ala Ile Arg Tyr Asp Arg Asp Gly Glu Pro Ser Gln Leu Val  
340 345 350

Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro  
35 355 360 365

Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro  
370 375 380

- 119 -

Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ser Ala Met Leu  
385                   390                   395                   400

Thr Phe Glu Ser Leu Ser Glu Thr Ala Glu Phe Ala Lys Lys Trp Val  
5                   405                   410                   415

Pro Phe Cys Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr  
420                   425                   430

10 Phe Ala Gln Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser Phe  
435                   440                   445

Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val  
450                   455                   460

15 Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Ile Pro Glu Glu Gly  
465                   470                   475                   480

Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Thr Arg Asp  
20                   485                   490                   495

His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp  
500                   505                   510

25 Thr Asp Gly Asn Glu Leu Pro Arg Leu Ile Tyr Val Ser Arg Glu Lys  
515                   520                   525

Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu  
530                   535                   540

30 Ile Arg Val Ser Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val  
545                   550                   555                   560

Asp Cys Asp His Tyr Phe Asn Asn Ser Lys Ala Ile Lys Glu Ala Met  
35                   565                   570                   575

Cys Phe Met Met Asp Pro Ala Ile Gly Lys Lys Cys Cys Tyr Val Gln  
580                   585                   590

- 120 -

Phe Pro Gln Arg Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn

595 600 605

Arg Asn Ile Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile

5 610 615 620

Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala

625 630 635 640

10 Leu Tyr Gly Tyr Asp Pro Val Leu Thr Glu Glu Asp Leu Glu Pro Asn

645 650 655

Ile Ile Val Lys Ser Cys Cys Gly Ser Arg Lys Lys Gly Lys Ser Ser

660 665 670

15

Lys Lys Tyr Asn Tyr Glu Lys Arg Arg Gly Ile Asn Arg Ser Asp Ser

675 680 685

Asn Ala Pro Leu Phe Asn Met Glu Asp Ile Asp Glu Gly Phe Glu Gly

20 690 695 700

Tyr Asp Asp Gln Arg Ser Ile Leu Met Ser Gln Arg Ser Val Gln Lys

705 710 715 720

25 Arg Phe Gly Gln Ser Pro Val Phe Ile Ala Ala Thr Phe Met Glu Gln

725 730 735

Gly Gly Ile Pro Pro Thr Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala

740 745 750

30

Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys

755 760 765

35 Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly

770 775 780

Phe Lys Met His Ala Arg Gly Trp Ile Ser Ile Tyr Cys Asn Pro Pro

785 790 795 800

- 121 -

Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu  
805 810 815

Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Ile Glu Ile Leu Leu Ser  
5 820 825 830

Arg His Cys Pro Ile Trp Tyr Gly Tyr His Gly Arg Leu Arg Leu Leu  
835 840 845

10 Glu Arg Ile Ala Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile  
850 855 860

Pro Leu Ile Ala Tyr Cys Ile Leu Pro Ala Phe Cys Leu Ile Thr Asp  
865 870 875 880

15 Arg Phe Ile Ile Pro Glu Ile Ser Asn Tyr Ala Ser Ile Trp Phe Ile  
885 890 895

Leu Leu Phe Ile Ser Ile Ala Val Thr Gly Ile Leu Glu Leu Arg Trp  
20 900 905 910

Ser Gly Val Ser Ile Glu Asp Trp Trp Arg Asn Glu Gln Phe Trp Val  
915 920 925

25 Ile Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu  
930 935 940

Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala  
945 950 955 960

30 Thr Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Ile Phe Lys Trp Thr  
965 970 975

Ala Leu Leu Ile Pro Pro Thr Thr Val Leu Leu Val Asn Leu Ile Gly  
35 980 985 990

Ile Val Ala Gly Val Ser Tyr Ala Val Asn Ser Gly Tyr Gln Ser Trp  
995 1000 1005

- 122 -

Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Leu Trp Val Ile Ala His

1010 1015 1020

Leu Tyr Pro Phe Leu Lys Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro

5 1025 1030 1035 1040

Thr Ile Val Ile Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu

1045 1050 1055

10 Leu Trp Val Arg Ile Asn Pro Phe Val Asp Ala Asn Pro Asn Ala Asn

1060 1065 1070

Asn Phe Asn Gly Lys Gly Gly Val Phe

1075 1080

15

(2) INFORMATION FOR SEQ ID NO:7:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3828 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

30 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Arabidopsis thaliana*
- (B) STRAIN: Columbia

35

(vii) IMMEDIATE SOURCE:

- (B) CLONE: *Ath-A*

(ix) FEATURE:

40 (A) NAME/KEY: CDS

- 123 -

(B) LOCATION: 239..3490

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

5	GTCGACACTA AGTGGATCCA AAGAATT CGC GGCGCGTCG ATACGGCTGC GAGAAGACGA	60	
	CAGAAAGGGGA TTGTCGATT C GGT TTT ATT TC GTCTCC TT CG TCTTCCACTC TT ACTAGTGC	120	
10	ATGCTCTGAA TCTGTATGTA ATGGGAGTT C AACAGTCTGG ATCCATTATC CTAGCCGGGT	180	
	CGGGTCAAGG TCTTTGAATA AGAGAGACAA TTCGTTTGA TT CCGGTGTAG AAGACATC	238	
	ATG AAT ACT GGT CGG CTC ATT GCT GGC TCT CAC AAC AGA AAC GAA	286	
15	Met Asn Thr Gly Gly Arg Leu Ile Ala Gly Ser His Asn Arg Asn Glu		
1	5	10	15
	TTC GTT CTC ATT AAC GCC GAT GAG AGT GCC AGA ATA CGA TCA GTA CAA	334	
	Phe Val Leu Ile Asn Ala Asp Glu Ser Ala Arg Ile Arg Ser Val Gln		
20	20 25 30		
	GAA CTG AGT GGG CAA ACA TGT CAA ATC TGT GGA GAT GAA ATC GAA TTA	382	
	Ser Leu Ser Gly Gin Thr Cys Gin Ile Cys Gly Asp Glu Ile Glu Leu		
25	35 40 45		
	ACG GTT AGC AGT GAG CTC TTT GTT GCT TGC AAC GAA TGC GCA TTC CCG	430	
	Thr Val Ser Ser Glu Leu Phe Val Ala Cys Asn Glu Cys Ala Phe Pro		
30	50 55 60		
	GTT TGT AGA CCA TGC TAT GAG TAT GAA CGT AGA GAA GGA AAT CAA GCT	478	
	Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Asn Gln Ala		
35	65 70 75 80		
	TGT CCT CAG TGC AAA ACT CGA TAC AAA AGG ATT AAA GGT AGT CCA CGG	526	
	Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Ile Lys Gly Ser Pro Arg		
40	85 90 95		
	GTT GAT GGA GAT GAT GAA GAA GAA GAC ATT GAT GAT CTT GAG TAT	574	
	Val Asp Gly Asp Asp Glu Glu Glu Asp Ile Asp Asp Leu Glu Tyr		
40	100	105	110

- 124 -

GAG TTT GAT CAT GGG ATG GAC CCT GAA CAT GCC GCT GAA GCC GCA CTC			622
Glu Phe Asp His Gly Met Asp Pro Glu His Ala Ala Glu Ala Ala Leu			
115	120	125	
5 TCT TCA CGC CTT AAC ACC GGT CGT GGT GGA TTG GAT TCA GCT CCA CCT			670
Ser Ser Arg Leu Asn Thr Gly Arg Gly Gly Leu Asp Ser Ala Pro Pro			
130	135	140	
10 GGC TCT CAG ATT CCT CTT TTG ACT TAT TGT GAT GAA GAT GCT GAT ATG			718
Gly Ser Gln Ile Pro Leu Leu Thr Tyr Cys Asp Glu Asp Ala Asp Met			
145	150	155	160
TAT TCT GAT CGT CAT GCT CTT ATC GTG CCT CCT TCA ACG GGA TAT GGG			766
Tyr Ser Asp Arg His Ala Leu Ile Val Pro Pro Ser Thr Gly Tyr Gly			
15	165	170	175
AAT CGC GTC TAT CCT GCA CCG TTT ACA GAT TCT TCT GCA CCT CCA CAG			814
Asn Arg Val Tyr Pro Ala Pro Phe Thr Asp Ser Ser Ala Pro Pro Gln			
180	185	190	
20			
GCG AGA TCA ATG GTT CCT CAG AAA GAT ATT GCG GAA TAT GGT TAT GGA			862
Ala Arg Ser Met Val Pro Gln Lys Asp Ile Ala Glu Tyr Tyr Gly			
195	200	205	
25 AGT GTT GCT TGG AAG GAC CGT ATG GAA GTT TGG AAG AGA CGA CAA GGC			910
Ser Val Ala Trp Lys Asp Arg Met Glu Val Trp Lys Arg Arg Gln Gly			
210	215	220	
30 GAA AAG CTT CAA GTC ATT AAG CAT GAA GGA GGA AAC AAT GGT CGA GGT			958
Glu Lys Leu Gln Val Ile Lys His Glu Gly Gly Asn Asn Gly Arg Gly			
225	230	235	240
35 TCC AAT GAT GAC GAC GAA CTA GAT GAT CCT GAC ATG CCT ATG ATG GAT			1006
Ser Asn Asp Asp Asp Glu Leu Asp Asp Pro Asp Met Pro Met Met Asp			
245	250	255	
GAA GGA AGA CAA CCT CTC TCA AGA AAG CTA CCT ATT CGT TCA AGC AGA			1054
Glu Gly Arg Gln Pro Leu Ser Arg Lys Leu Pro Ile Arg Ser Ser Arg			
260	265	270	

- 125 -

ATA AAT CCT TAC AGG ATG TTA ATT CTG TGT CGC CTC GCG ATT CTT GGT Ile Asn Pro Tyr Arg Met Leu Ile Leu Cys Arg Leu Ala Ile Leu Gly 275                    280                    285	1102
5 CTT TTC TTT CAT TAT AGA ATT CTC CAT CCA GTC AAT GAT GCA TAT GGA Leu Phe Phe His Tyr Arg Ile Leu His Pro Val Asn Asp Ala Tyr Gly 290                    295                    300	1150
10 TTA TGG TTA ACG TCA GTT ATA TGC GAA ATA TGG TTT GCA GTG TCT TGG Leu Trp Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Val Ser Trp 305                    310                    315                    320	1198
15 ATT CTT GAT CAA TTC CCC AAA TGG TAT CCT ATA GAA CGT GAA ACA TAC Ile Leu Asp Gln Phe Pro Lys Trp Tyr Pro Ile Glu Arg Glu Thr Tyr 325                    330                    335	1246
20 CTC GAT AGA CTC TCT CTC AGG TAC GAG AAG GAA GGA AAA CCG TCA GGA Leu Asp Arg Leu Ser Leu Arg Tyr Glu Lys Glu Gly Lys Pro Ser Gly 340                    345                    350	1294
25 TTA GCA CCT GTT GAT GTT TTT GTT AGT ACA GTG GAT CCG TTG AAA GAG Leu Ala Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu 355                    360                    365	1342
30 CCC CCC TTG ATT ACA GCA AAC ACA GTT CTT TCC ATT CTA GCA GTT GAT Pro Pro Leu Ile Thr Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp 370                    375                    380	1390
35 TAT CCT GTG GAT AAG GTT GCG TGT TAT GTA TCA AAC AAT GGT GCA GCT Tyr Pro Val Asp Lys Val Ala Cys Tyr Val Ser Asn Asn Gly Ala Ala 385                    390                    395                    400	1438
40 ATG CTT ACA TTT GAA GCT CTC TCT GAT ACA GCT GAT TTT GCT ACA AAA Met Leu Thr Phe Glu Ala Leu Ser Asp Thr Ala Asp Phe Ala Thr Lys 405                    410                    415	1486
TGG GTT CCT TTT TGT AAG AAG TTT AAT ATC GAG CCA CGA GCT CCT GAG Trp Val Pro Phe Cys Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu 420                    425                    430	1534

- 126 -

TGG TAT TTT TCT CAG AAG ATG GAT TAC CTG AAG AAC AAA GTT CAT CCT			1582
Trp Tyr Phe Ser Gln Lys Met Asp Tyr Leu Lys Asn Lys Val His Pro			
435	440	445	
 5 GCT TTT GTC AGG GAA CGT CGT GCT ATG AAG AGA GAT TAT GAA GAG TTT			1630
Ala Phe Val Arg Glu Arg Arg Ala Met Lys Arg Asp Tyr Glu Glu Phe			
450	455	460	
 10 AAA GTG AAG ATA AAT GCA CTG GTT GCT ACT GCA CAG AAA GTG CCT GAG			1678
Lys Val Lys Ile Asn Ala Leu Val Ala Thr Ala Gln Lys Val Pro Glu			
465	470	475	480
 15 GAA CGT TGG ACT ATG CAA GAT GGA ACT CCT TGG CCT GGA AAC AAC GTC			1726
Glu Arg Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val			
485	490	495	
 20 CGT GAC CAT CCT GGA ATG ATT CAG GTG TTC TTG GGT CAT AGT GGA GTT			1774
Arg Asp His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Val			
500	505	510	
 25 CGT GAT ACG GAT GGT AAT GAG TTA CCA CGT CTA GTG TAT GTT TCT CGT			1822
Arg Asp Thr Asp Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg			
515	520	525	
 30 GAG AAG CGG CCT GGA TTT GAT CAC CAC AAG AAA GCT GGA GCT ATG AAT			1870
Glu Lys Arg Pro Gly Phe Asp His His Lys Lys Ala Gly Ala Met Asn			
530	535	540	
 35 TCC TTG ATC CGA GTC TCT GCT GTT CTA TCA AAC GCT CCT TAC CTT CTT			1918
Ser Leu Ile Arg Val Ser Ala Val Leu Ser Asn Ala Pro Tyr Leu Leu			
545	550	555	560
 40 AAT GTC GAT TGT GAT CAC TAC ATC AAC AAC AGC AAA GCA ATT AGA GAA			1966
Asn Val Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Arg Glu			
565	570	575	
 45 TCT ATG TGT TTC ATG ATG GAC CCG CAA TCG GGA AAG AAA GTT TGT TAT			2014
Ser Met Cys Phe Met Met Asp Pro Gln Ser Gly Lys Lys Val Cys Tyr			
580	585	590	

- 127 -

GTT CAG TTT CCG CAG AGA TTT GAT GGG ATT GAT AGA CAT GAT AGA TAC			2062
Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr			
595	600	605	
 5 TCA AAC CGT AAC GTT GTG TTC TTT GAT ATT AAC ATG AAA GGT CTT GAT			2110
Ser Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp			
610	615	620	
 GGG ATA CAA GGA CCG ATA TAT GTC GGG ACA GGT TGT GTG TTT AGA AAA			2158
10 Gly Ile Gln Gly Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Lys			
625	630	635	640
 CAG GCT CTT TAT GGT TTT GAT GCA CCA AAG AAG AAG AAA CCA CCA GGC			2206
Gln Ala Leu Tyr Gly Phe Asp Ala Pro Lys Lys Lys Pro Pro Gly			
15	645	650	655
 AAA ACC TGT AAC TGT TGG CCT AAA TGG TGT TGT TTG TGT GGG TTG			2254
Lys Thr Cys Asn Cys Trp Pro Lys Trp Cys Cys Leu Cys Cys Gly Leu			
660	665	670	
20 AGA AAG AAG AGT AAA ACG AAA GCC ACA GAT AAG AAA ACT AAC ACT AAA			2302
Arg Lys Lys Ser Lys Thr Lys Ala Thr Asp Lys Lys Thr Asn Thr Lys			
675	680	685	
 25 GAG ACT TCA AAG CAG ATT CAT GCG CTA GAG AAT GTC GAC GAA GGT GTT			2350
Glu Thr Ser Lys Gln Ile His Ala Leu Glu Asn Val Asp Glu Gly Val			
690	695	700	
 ATC GTC CCA GTG TCA AAT GTT GAG AAG AGA TCT GAA GCA ACA CAA TTG			2398
30 Ile Val Pro Val Ser Asn Val Glu Lys Arg Ser Glu Ala Thr Gln Leu			
705	710	715	720
 AAA TTG GAG AAG TTT GGA CAA TCT CCG GTT TTC GTT GCC TCT GCT			2446
Lys Leu Glu Lys Lys Phe Gly Gln Ser Pro Val Phe Val Ala Ser Ala			
35	725	730	735
 GTT CTA CAG AAC GGT GGA GTT CCC CGT AAC GCA AGC CCC GCA TGT TTG			2494
Val Leu Gln Asn Gly Gly Val Pro Arg Asn Ala Ser Pro Ala Cys Leu			
740	745	750	

- 128 -

TTA AGA GAA GCC ATT CAA GTT ATT AGC TGC GGG TAC CAA GAT AAA ACC Leu Arg Glu Ala Ile Gln Val Ile Ser Cys Gly Tyr Gln Asp Lys Thr	755	760	765	2542
5 GAA TGG GGA AAA GAG ATC GGG TGG ATT TAT GGA TCG GTG ACT GAA GAT Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp	770	775	780	2590
10 ATC CTG ACG GGT TTC AAG ATG CAT TGC CAT GGA TGG AGA TCT GTG TAC Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser Val Tyr	785	790	795	800
15 TGT ATG CCT AAG CGT GCA GCT TTT AAA GGA TCT GCT CCT ATT AAC TTG Cys Met Pro Lys Arg Ala Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu	805	810	815	2638
20 TCA GAT CGT CTT CAT CAA GTT CTA CGT TGG GCT CTT GGC TCT GTA GAG Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu	820	825	830	2686
25 ATT TTC TTG AGC AGA CAT TGT CCG ATA TGG TAT GGT TAT GGT GGT GGT Ile Phe Leu Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Gly Gly Gly	835	840	845	2734
30 TTA AAA TGG TTG GAG AGA TTC TCT TAC ATC AAC TCT GTC GTC TAT CCT Leu Lys Trp Leu Glu Arg Phe Ser Tyr Ile Asn Ser Val Val Tyr Pro	850	855	860	2782
35 TGG ACT TCA CTT CCA TTG ATC GTC TAT TGT TCT CTC CCC GCG GTT TGT Trp Thr Ser Leu Pro Leu Ile Val Tyr Cys Ser Leu Pro Ala Val Cys	865	870	875	2878
40 TTA CTC ACA GGA AAA TTC ATC GTC CCT GAG ATA AGC AAC TAC GCA GGT Leu Leu Thr Gly Lys Phe Ile Val Pro Glu Ile Ser Asn Tyr Ala Gly	885	890	895	2926
ATA CTC TTC ATG CTC ATG TTC ATA TCC ATA GCA GTA ACT GGA ATC CTC Ile Leu Phe Met Leu Met Phe Ile Ser Ile Ala Val Thr Gly Ile Leu	900	905	910	2974

- 129 -

GAA ATG CAA TGG GGA GGT GTC GGA ATC GAT GAT TGG TGG AGA AAC GAG			3022
Glu Met Gln Trp Gly Gly Val Gly Ile Asp Asp Trp Trp Arg Asn Glu			
915	920	925	
 5 CAG TTT TGG GTA ATC GGA GGG GCC TCC TCG CAT CTA TTT GCT CTG TTT			3070
Gln Phe Trp Val Ile Gly Gly Ala Ser Ser His Leu Phe Ala Leu Phe			
930	935	940	
 CAA GGT TTG CTC AAA GTT CTA GCC GGA GTT AAC ACG AAT TTC ACA GTC			3118
10 Gln Gly Leu Leu Lys Val Leu Ala Gly Val Asn Thr Asn Phe Thr Val			
945	950	955	960
 ACT TCA AAA GCA GCA GAC GAT GGA GCT TTC TCT GAG CTT TAC ATC TTC			3166
Thr Ser Lys Ala Ala Asp Asp Gly Ala Phe Ser Glu Leu Tyr Ile Phe			
15 965	970	975	
 AAG TGG ACA ACT TTG TTG ATT CCT CCG ACA ACA CTT CTG ATC ATT AAC			3214
Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Ile Ile Asn			
980	985	990	
20 ATC ATT GGA GTT ATT GTC GGC GTT TCT GAT GCC ATT AGC AAT GGC TAT			3262
Ile Ile Gly Val Ile Val Gly Val Ser Asp Ala Ile Ser Asn Gly Tyr			
995	1000	1005	
 25 GAC TCA TGG GGA CCT CTC TTT GGG AGA CTT TTC GCT CTT TGG GTC			3310
Asp Ser Trp Gly Pro Leu Phe Gly Arg Leu Phe Phe Ala Leu Trp Val			
1010	1015	1020	
 ATT GTT CAT TTA TAC CCA TTC CTC AAG GGA ATG CTT GGG AAG CAA GAC			3358
30 Ile Val His Leu Tyr Pro Phe Leu Lys Gly Met Leu Gly Lys Gln Asp			
1025	1030	1035	1040
 AAA ATG CCT ACG ATT ATT GTG GTC TGG TCT ATT CTT CTA GCT TCG ATC			3406
Lys Met Pro Thr Ile Ile Val Val Trp Ser Ile Leu Leu Ala Ser Ile			
35 1045	1050	1055	
 TTG ACA CTC TTG TGG GTC AGA ATT AAC CCG TTT GTG GCT AAA GGG GGA			3454
Leu Thr Leu Leu Trp Val Arg Ile Asn Pro Phe Val Ala Lys Gly Gly			
1060	1065	1070	

- 130 -

CCA GTG TTG GAG ATC TGT GGT CTG AAT TGT GGA AAC TAAGATCCTC	3500
Pro Val Leu Glu Ile Cys Gly Leu Asn Cys Gly Asn	
1075	1080
5 AGTGAAAGAA GAGCAAAGGA GTTTGTGTTG GAGCTTGGA AGCAAATGTG TTGATGATGA	3560
TGCAAGTGTG TTTGTAGACA AAGATGTGCA GTTTTACTT TTTACGACTT GTTAAACCTT	3620
10 TTTGTTACC CCTAAATTAA TTCTTTGTT ATCATGGTAA TACTAATAGA ATTGTTTGT	3680
TTTCCTTTT ACATGTACTT TTAGTTATT CGTAGTTATT GTATAAATCT GATAACGATC	3740
ATATATACAC ACTTTGTTAA CAAAAAAA AAAAAAAA AAAAAAAA AAAGCGGCCG	3800
15 CTCGAATTGT CGACGCGGCC GCGAATTG	3828

**20 (2) INFORMATION FOR SEQ ID NO:8:**

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1084 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

30 Met Asn Thr Gly Gly Arg Leu Ile Ala Gly Ser His Asn Arg Asn Glu  
1 5 10 15

Phe	Val	Leu	Ile	Asn	Ala	Asp	Glu	Ser	Ala	Arg	Ile	Arg	Ser	Val	Gln
35		20					25					30			

Glu Leu Ser Gly Gln Thr Cys Gln Ile Cys Gly Asp Glu Ile Glu Leu  
35 40 45

- 131 -

Thr Val Ser Ser Glu Leu Phe Val Ala Cys Asn Glu Cys Ala Phe Pro  
50 55 60

Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Asn Gln Ala  
5 65 70 75 80

Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Ile Lys Gly Ser Pro Arg  
85 90 95

10 Val Asp Gly Asp Asp Glu Glu Glu Asp Ile Asp Asp Leu Glu Tyr  
100 105 110

Glu Phe Asp His Gly Met Asp Pro Glu His Ala Ala Glu Ala Ala Leu  
115 120 125

15 Ser Ser Arg Leu Asn Thr Gly Arg Gly Gly Leu Asp Ser Ala Pro Pro  
130 135 140

Gly Ser Gln Ile Pro Leu Leu Thr Tyr Cys Asp Glu Asp Ala Asp Met  
20 145 150 155 160

Tyr Ser Asp Arg His Ala Leu Ile Val Pro Pro Ser Thr Gly Tyr Gly  
165 170 175

25 Asn Arg Val Tyr Pro Ala Pro Phe Thr Asp Ser Ser Ala Pro Pro Gln  
180 185 190

Ala Arg Ser Met Val Pro Gln Lys Asp Ile Ala Glu Tyr Gly Tyr Gly  
195 200 205

30 Ser Val Ala Trp Lys Asp Arg Met Glu Val Trp Lys Arg Arg Gln Gly  
210 215 220

Glu Lys Leu Gln Val Ile Lys His Glu Gly Gly Asn Asn Gly Arg Gly  
35 225 230 235 240

Ser Asn Asp Asp Asp Glu Leu Asp Asp Pro Asp Met Pro Met Met Asp  
245 250 255

- 132 -

Glu Gly Arg Gln Pro Leu Ser Arg Lys Leu Pro Ile Arg Ser Ser Arg  
260 265 270

Ile Asn Pro Tyr Arg Met Leu Ile Leu Cys Arg Leu Ala Ile Leu Gly  
5 275 280 285

Leu Phe Phe His Tyr Arg Ile Leu His Pro Val Asn Asp Ala Tyr Gly  
290 295 300

10 Leu Trp Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Val Ser Trp  
305 310 315 320

Ile Leu Asp Gln Phe Pro Lys Trp Tyr Pro Ile Glu Arg Glu Thr Tyr  
325 330 335

15 Leu Asp Arg Leu Ser Leu Arg Tyr Glu Lys Glu Gly Lys Pro Ser Gly  
340 345 350

Leu Ala Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu  
20 355 360 365

.Pro Pro Leu Ile Thr Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp  
370 375 380

25 Tyr Pro Val Asp Lys Val Ala Cys Tyr Val Ser Asn Asn Gly Ala Ala  
385 390 395 400

Met Leu Thr Phe Glu Ala Leu Ser Asp Thr Ala Asp Phe Ala Thr Lys  
405 410 415

30 Trp Val Pro Phe Cys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu  
420 425 430

Trp Tyr Phe Ser Gln Lys Met Asp Tyr Leu Lys Asn Lys Val His Pro  
35 435 440 445

Ala Phe Val Arg Glu Arg Arg Ala Met Lys Arg Asp Tyr Glu Glu Phe  
450 455 460

- 133 -

Lys Val Lys Ile Asn Ala Leu Val Ala Thr Ala Gln Lys Val Pro Glu  
465 470 475 480

Glu Arg Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val  
5 485 490 495

Arg Asp His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Val  
500 505 510

10 Arg Asp Thr Asp Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg  
515 520 525

Glu Lys Arg Pro Gly Phe Asp His His Lys Lys Ala Gly Ala Met Asn  
530 535 540

15 Ser Leu Ile Arg Val Ser Ala Val Leu Ser Asn Ala Pro Tyr Leu Leu  
545 550 555 560

Asn Val Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Arg Glu  
20 565 570 575

Ser Met Cys Phe Met Met Asp Pro Gln Ser Gly Lys Lys Val Cys Tyr  
580 585 590

25 Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr  
595 600 605

Ser Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp  
610 615 620

30 Gly Ile Gln Gly Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Lys  
625 630 635 640

Gln Ala Leu Tyr Gly Phe Asp Ala Pro Lys Lys Lys Pro Pro Gly  
35 645 650 655

Lys Thr Cys Asn Cys Trp Pro Lys Trp Cys Cys Leu Cys Cys Gly Leu  
660 665 670

- 134 -

Arg Lys Lys Ser Lys Thr Lys Ala Thr Asp Lys Lys Thr Asn Thr Lys  
675                    680                    685

Glu Thr Ser Lys Gln Ile His Ala Leu Glu Asn Val Asp Glu Gly Val  
5        690                    695                    700

Ile Val Pro Val Ser Asn Val Glu Lys Arg Ser Glu Ala Thr Gln Leu  
705                    710                    715                    720

10 Lys Leu Glu Lys Lys Phe Gly Gln Ser Pro Val Phe Val Ala Ser Ala  
725                    730                    735

Val Leu Gln Asn Gly Gly Val Pro Arg Asn Ala Ser Pro Ala Cys Leu  
740                    745                    750

15

Leu Arg Glu Ala Ile Gln Val Ile Ser Cys Gly Tyr Gln Asp Lys Thr  
755                    760                    765

Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp  
20        770                    775                    780

Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser Val Tyr  
785                    790                    795                    800

25 Cys Met Pro Lys Arg Ala Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu  
805                    810                    815

Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu  
820                    825                    830

30

Ile Phe Leu Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Gly Gly  
835                    840                    845

Leu Lys Trp Leu Glu Arg Phe Ser Tyr Ile Asn Ser Val Val Tyr Pro  
35        850                    855                    860

Trp Thr Ser Leu Pro Leu Ile Val Tyr Cys Ser Leu Pro Ala Val Cys  
865                    870                    875                    880

- 135 -

Leu Leu Thr Gly Lys Phe Ile Val Pro Glu Ile Ser Asn Tyr Ala Gly  
885 890 895

Ile Leu Phe Met Leu Met Phe Ile Ser Ile Ala Val Thr Gly Ile Leu  
5 900 905 910

Glu Met Gln Trp Gly Gly Val Gly Ile Asp Asp Trp Trp Arg Asn Glu  
915 920 925

10 Gln Phe Trp Val Ile Gly Gly Ala Ser Ser His Leu Phe Ala Leu Phe  
930 935 940

Gln Gly Leu Leu Lys Val Leu Ala Gly Val Asn Thr Asn Phe Thr Val  
945 950 955 960

15

Thr Ser Lys Ala Ala Asp Asp Gly Ala Phe Ser Glu Leu Tyr Ile Phe  
965 970 975

Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Ile Ile Asn  
20 980 985 990

Ile Ile Gly Val Ile Val Gly Val Ser Asp Ala Ile Ser Asn Gly Tyr  
995 1000 1005

25 Asp Ser Trp Gly Pro Leu Phe Gly Arg Leu Phe Phe Ala Leu Trp Val  
1010 1015 1020

Ile Val His Leu Tyr Pro Phe Leu Lys Gly Met Leu Gly Lys Gln Asp  
1025 1030 1035 1040

30

Lys Met Pro Thr Ile Ile Val Val Trp Ser Ile Leu Leu Ala Ser Ile  
1045 1050 1055

Leu Thr Leu Leu Trp Val Arg Ile Asn Pro Phe Val Ala Lys Gly Gly  
35 1060 1065 1070

Pro Val Leu Glu Ile Cys Gly Leu Asn Cys Gly Asn  
1075 1080

40

- 136 -

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 3614 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

15 (iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- 15 (A) ORGANISM: *Arabidopsis thaliana*
- (B) STRAIN: Columbia

20 (vii) IMMEDIATE SOURCE:

- (B) CLONE: Ath-B

25 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 217..3411

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAATTCGCGG CCGCGTCGAC TACGGCTGCG AGAAGACGAC AGAAGGGAT CCCAAGATTG	60
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TCCTCTTCGT CTTCCCTTATA AACTATCTCT CTGTAGAGAA GAAAGCTTGG ATCCAGATTG	120
--	-----

30 AGAGAGATTC AGAGAGCCAC ATCACCAACAC TCCATCTTCA GATCTCATGA TTTGAACATAT	180
--	-----

TCCGACGTTT CGGTGTTGGA AGCAACTAAG TGACAA ATG GAA TCC GAA GGA GAA	234
---	-----

Met Glu Ser Glu Gly Glu	
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35 1 5	
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ACC GCG GGA AAG CCG ATG AAG AAC ATT GTT CCG CAG ACT TGC CAG ATC	282
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Thr Ala Gly Lys Pro Met Lys Asn Ile Val Pro Gln Thr Cys Gln Ile	
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10 15 20	
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40	
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- 137 -

TGT AGT GAC AAT GTT GGC AAG ACT GTT GAT GGA GAT CGT TTT GTG GCT		330
Cys Ser Asp Asn Val Gly Lys Thr Val Asp Gly Asp Arg Phe Val Ala		
25	30	35
5 TGT GAT ATT TGT TCA TTC CCA GTT TGT CGG CCT TGC TAC GAG TAT GAG		378
Cys Asp Ile Cys Ser Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu		
40	45	50
10 AGG AAA GAT GGG AAT CAA TCT TGT CCT CAG TGC AAA ACC AGA TAC AAG		426
Arg Lys Asp Gly Asn Gln Ser Cys Pro Gln Cys Lys Thr Arg Tyr Lys		
55	60	65
15 AGG CTC AAA GGT AGT CCT GCT ATT CCT GGT GAT AAA GAC GAG GAT GGC		474
Arg Leu Lys Gly Ser Pro Ala Ile Pro Gly Asp Lys Asp Glu Asp Gly		
75	80	85
20 TTA GCT GAT GAA GGT ACT GTT GAG TTC AAC TAC CCT CAG AAG GAG AAA		522
Leu Ala Asp Glu Gly Thr Val Glu Phe Asn Tyr Pro Gln Lys Glu Lys		
90	95	100
25 ATT TCA GAG CGG ATG CTT GGT TGG CAT CTT ACT CGT GGG AAG GGA GAG		570
Ile Ser Glu Arg Met Leu Gly Trp His Leu Thr Arg Gly Lys Gly Glu		
105	110	115
30 GAA ATG GGG GAA CCC CAG TAT GAT AAA GAG GTC TCT CAC AAT CAT CTT		618
Glu Met Gly Glu Pro Gln Tyr Asp Lys Glu Val Ser His Asn His Leu		
120	125	130
35 CCT CGT CTC ACG AGC AGA CAA GAT ACT TCA GGA GAG TTT TCT GCT GCC		666
Pro Arg Leu Thr Ser Arg Gln Asp Thr Ser Gly Glu Phe Ser Ala Ala		
135	140	145
150		
40 TCA CCT GAA CGC CTC TCT GTA TCT ACT ATC GCT GGG GGA AAG CGC		714
Ser Pro Glu Arg Leu Ser Val Ser Ser Thr Ile Ala Gly Gly Lys Arg		
155	160	165
45 CTT CCC TAT TCA TCA GAT GTC AAT CAA TCA CCA AAT AGA AGG ATT GTG		762
Leu Pro Tyr Ser Ser Asp Val Asn Gln Ser Pro Asn Arg Arg Ile Val		
170	175	180

- 138 -

GAT CCT GTT GGA CTC GGG AAT GTA GCT TGG AAG GAG AGA GTT GAT GGC			810
Asp Pro Val Gly Leu Gly Asn Val Ala Trp Lys Glu Arg Val Asp Gly			
185	190	195	
 5 TGG AAA ATG AAG CAA GAG AAG AAT ACT GGT CCT GTC AGC ACG CAG GCT			858
Trp Lys Met Lys Gln Glu Lys Asn Thr Gly Pro Val Ser Thr Gln Ala			
200	205	210	
 10 GCT TCT GAA AGA GGT GGA GTA GAT ATT GAT GCC AGC ACA GAT ATC CTA			906
Ala Ser Glu Arg Gly Val Asp Ile Asp Ala Ser Thr Asp Ile Leu			
215	220	225	230
 15 GCA GAT GAG GCT CTG CTG AAT GAC GAA GCG AGG CAG CTT CTG TCA AGG			954
Ala Asp Glu Ala Leu Leu Asn Asp Glu Ala Arg Gln Leu Leu Ser Arg			
235	240	245	
 20 AAA GTT TCA ATT CCT TCA TCA CGG ATC AAT CCT TAC AGA ATG GTT ATT			1002
Lys Val Ser Ile Pro Ser Ser Arg Ile Asn Pro Tyr Arg Met Val Ile			
255	260		
 25 ATG CTG CGG CTT GTT ATC CTT TGT CTC TTC TTG CAT TAC CGT ATA ACA			1050
Met Leu Arg Leu Val Ile Leu Cys Leu Phe Leu His Tyr Arg Ile Thr			
265	270	275	
 30 AAC CCA GTG CCA AAT GCC TTT GCT CTA TGG CTG GTC TCT GTG ATA TGT			1098
Asn Pro Val Pro Asn Ala Phe Ala Leu Trp Leu Val Ser Val Ile Cys			
280	285	290	
 35 GAG ATC TGG TTT GCC TTA TCC TGG ATT TTG GAT CAG TTT CCC AAG TGG			1146
Glu Ile Trp Phe Ala Leu Ser Trp Ile Leu Asp Gln Phe Pro Lys Trp			
295	300	305	310
 40 TTT CCT GTG AAC CGT GAA ACC TAC CTC GAC AGG CTT GCT TTA AGA TAT			1194
Phe Pro Val Asn Arg Glu Thr Tyr Leu Asp Arg Leu Ala Leu Arg Tyr			
315	320	325	
 45 GAT CGT GAA GGT GAG CCA TCA CAG TTA GCT GCT GTT GAC ATT TTC GTG			1242
Asp Arg Glu Gly Glu Pro Ser Gln Leu Ala Ala Val Asp Ile Phe Val			
330	335	340	

- 139 -

	AGT ACT GTT GAC CCC TTG AAG GAG CCA CCC CTT GTG ACA GCC AAC ACA		1290
Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Val Thr Ala Asn Thr			
345	350	355	
5 GTG CTC TCT ATT CTG GCT GTT GAC TAC CCA GTT GAC AAG GTG TCC TGT			1338
Val Leu Ser Ile Leu Ala Val Asp Tyr Pro Val Asp Lys Val Ser Cys			
360	365	370	
TAT GTT TCT GAT GAT GGT GCT GCT ATG TTA TCA TTT GAA TCA CTT GCA			1386
10 Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Ser Phe Glu Ser Leu Ala			
375	380	385	390
GAA ACA TCA GAG TTT GCT CGT AAA TGG GTA CCA TTT TGC AAG AAA TAT			1434
Glu Thr Ser Glu Phe Ala Arg Lys Trp Val Pro Phe Cys Lys Lys Tyr			
15 395	400	405	
AGC ATA GAG CCT CGT GCA CCA GAA TGG TAC TTT GCT GCG AAA ATA GAT			1482
Ser Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Ala Ala Lys Ile Asp			
410	415	420	
20 TAC TTG AAG GAT AAA GTT CAG ACA TCA TTT GTC AAA GAT CGT AGA GCT			1530
Tyr Leu Lys Asp Lys Val Gln Thr Ser Phe Val Lys Asp Arg Arg Ala			
425	430	435	
25 ATG AAG AGG GAA TAT GAG GAA TTT AAA ATC CGA ATC AAT GCA CTT GTT			1578
Met Lys Arg Glu Tyr Glu Glu Phe Lys Ile Arg Ile Asn Ala Leu Val			
440	445	450	
TCC AAA GCC CTA AAA TGT CCT GAA GAA GGG TGG GTT ATG CAA GAT GGC			1626
30 Ser Lys Ala Leu Lys Cys Pro Glu Glu Gly Trp Val Met Gln Asp Gly			
455	460	465	470
ACA CCG TGG CCT GGA AAT AAT ACA GGG GAC CAT CCA GGA ATG ATC CAG			1674
Thr Pro Trp Pro Gly Asn Asn Thr Gly Asp His Pro Gly Met Ile Gln			
35 475	480	485	
GTC TTC TTA GGG CAA AAT GGT GGA CTT GAT GCA GAG GGC AAT GAG CTC			1722
Val Phe Leu Gly Gln Asn Gly Gly Leu Asp Ala Glu Gly Asn Glu Leu			
490	495	500	

- 140 -

	CCG CGT TTG GTA TAT GTT TCT CGA GAA AAG CGA CCA GGA TTC CAG CAC		1770
	Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe Gln His		
	505	510	515
5	CAC AAA AAG GCT GGT GCT ATG AAT GCA CTG GTG AGA GTT TCA GCA GTT		1818
	His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg Val Ser Ala Val		
	520	525	530
10	CTT ACC AAT GGA CCT TTC ATC TTG AAT CTT GAT TGT GAT CAT TAC ATA		1866
	Leu Thr Asn Gly Pro Phe Ile Leu Asn Leu Asp Cys Asp His Tyr Ile		
	535	540	545
15	AAT AAC ACC AAA GCC TTA AGA GAA GCA ATG TGC TTC CTG ATG GAC CCA		1914
	Asn Asn Ser Lys Ala Leu Arg Glu Ala Met Cys Phe Leu Met Asp Pro		
	555	560	565
20	AAC CTC GGG AAG CAA GTT TGT TAT GTT CAG TTC CCA CAA AGA TTT GAT		1962
	Asn Leu Gly Lys Gln Val Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp		
	570	575	580
25	GGT ATC GAT AAG AAC GAT AGA TAT GCT AAT CGT AAT ACC GTG TTC TTT		2010
	Gly Ile Asp Lys Asn Asp Arg Tyr Ala Asn Arg Asn Thr Val Phe Phe		
	585	590	595
30	GAT ATT AAC TTG AGA GGT TTA GAT GGG ATT CAA GGA CCT GTA TAT GTC		2058
	Asp Ile Asn Leu Arg Gly Leu Asp Gly Ile Gln Gly Pro Val Tyr Val		
	600	605	610
35	GGA ACT GGA TGT GTT TTC AAC AGA ACA GCA TTA TAC GGT TAT GAA CCT		2106
	Gly Thr Gly Cys Val Phe Asn Arg Thr Ala Leu Tyr Gly Tyr Glu Pro		
	615	620	625
	CCA ATA AAA GTA AAA CAC AAG AAG CCA AGT CTT TTA TCT AAG CTC TGT		2154
	Pro Ile Lys Val Lys His Lys Lys Pro Ser Leu Leu Ser Lys Leu Cys		
	635	640	645
40	GGT GGA TCA AGA AAG AAG AAT TCC AAA GCT AAG AAA GAG TCG GAC AAA		2202
	Gly Gly Ser Arg Lys Lys Asn Ser Lys Ala Lys Lys Glu Ser Asp Lys		
	650	655	660

- 141 -

AAG AAA TCA GGC AGG CAT ACT GAC TCA ACT GTT CCT GTA TTC AAC CTC Lys Lys Ser Gly Arg His Thr Asp Ser Thr Val Pro Val Phe Asn Leu	2250
665                   670                   675	
 5 GAT GAC ATA GAA GAG GGA GTT GAA GGT GCT GGT TTT GAT GAT GAA AAG Asp Asp Ile Glu Glu Gly Val Glu Gly Ala Gly Phe Asp Asp Glu Lys	2298
680                   685                   690	
 10 GCG CTC TTA ATG TCG CAA ATG AGC CTG GAG AAG CGA TTT GGA CAG TCT Ala Leu Leu Met Ser Gln Met Ser Leu Glu Lys Arg Phe Gly Gln Ser	2346
695                   700                   705                   710	
 15 GCT GTT TTT GTT GCT TCT ACC CTA ATG GAA AAT GGT GGT GTT CCT CCT Ala Val Phe Val Ala Ser Thr Leu Met Glu Asn Gly Gly Val Pro Pro	2394
715                   720                   725	
 20 TCA GCA ACT CCA GAA AAC TTT CTC AAA GAG GCT ATC CAT GTC ATT AGT Ser Ala Thr Pro Glu Asn Phe Leu Lys Glu Ala Ile His Val Ile Ser	2442
730                   735                   740	
 25 TGT GGT TAT GAG GAT AAG TCA GAT TGG GGA ATG GAG ATT GGA TGG ATC Cys Gly Tyr Glu Asp Lys Ser Asp Trp Gly Met Glu Ile Gly Trp Ile	2490
745                   750                   755	
 30 TAT GGT TCT GTG ACA GAA GAT ATT CTG ACT GGG TTC AAA ATG CAT GCC Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala	2538
760                   765                   770	
 35 CGT GGA TGG CGA TCC ATT TAC TGC ATG CCT AAG CTT CCA GCT TTC AAG Arg Gly Trp Arg Ser Ile Tyr Cys Met Pro Lys Leu Pro Ala Phe Lys	2586
775                   780                   785                   790	
 40 GGT TCT GCT CCT ATC AAT CTT TCA GAT CGT CTG AAC CAA GTG CTG AGG Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg	2634
795                   800                   805	
 45 TGG GCT TTA GGT TCA GTT GAG ATT CTC TTC AGT CGG CAT TGT CCT ATA Trp Ala Leu Gly Ser Val Glu Ile Leu Phe Ser Arg His Cys Pro Ile	2682
810                   815                   820	

- 142 -

TGG TAT GGT TAC AAT GGG AGG CTA AAA TTT CCT GAG AGG TTT GCG TAT			2730
Trp Tyr Gly Tyr Asn Gly Arg Leu Lys Phe Leu Glu Arg Phe Ala Tyr			
825	830	835	
5 GTG AAC ACC ACC ATC TAC CCT ATC ACC TCC ATT CCT CTT CTC ATG TAT			2778
Val Asn Thr Thr Ile Tyr Pro Ile Thr Ser Ile Pro Leu Leu Met Tyr			
840	845	850	
TGT ACA TTG CTA GCC GTT TGT CTC TTC ACC AAC CAG TTT ATT ATT CCT			2826
10 Cys Thr Leu Leu Ala Val Cys Leu Phe Thr Asn Gln Phe Ile Ile Pro			
855	860	865	870
CAG ATT AGT AAC ATT GCA AGT ATA TGG TTT CTG TCT CTC TTT CTC TCC			2874
Gln Ile Ser Asn Ile Ala Ser Ile Trp Phe Leu Ser Leu Phe Leu Ser			
15	875	880	885
ATT TTC GCC ACG GGT ATA CTA GAA ATG AGG TGG AGT GGC GTA GGC ATA			2922
Ile Phe Ala Thr Gly Ile Leu Glu Met Arg Trp Ser Gly Val Gly Ile			
890	895	900	
20			
GAC GAA TGG TGG AGA AAC GAG CAG TTT TGG GTC ATT GGT GGA GTA TCC			2970
Asp Glu Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser			
905	910	915	
25 GCT CAT TTA TTC GCT GTG TTT CAA GGT ATC CTC AAA GTC CTT GCC GGT			3018
Ala His Leu Phe Ala Val Phe Gln Gly Ile Leu Lys Val Leu Ala Gly			
920	925	930	
ATT GAC ACA AAC TTC ACA GTT ACC TCA AAA GCT TCA GAT GAA GAC GGA			3066
30 Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp Gly			
935	940	945	950
GAC TTT GCT GAG CTC TAC TTG TTC AAA TGG ACA ACA CTT CTG ATT CCG			3114
Asp Phe Ala Glu Leu Tyr Leu Phe Lys Trp Thr Thr Leu Leu Ile Pro			
35	955	960	965
CCA ACG ACG CTG CTC ATT GTA AAC TTA GTG GGA GTT GTT GCA GGA GTC			3162
Pro Thr Thr Leu Leu Ile Val Asn Leu Val Gly Val Val Ala Gly Val			
970	975	980	

- 143 -

TCT TAT GCT ATC AAC AGT GGA TAC CAA TCA TGG GGA CCA CTC TTT GGT	3210		
Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly			
985	990	995	
5 AAG TTG TTC TTT GCC TTC TGG GTG ATT GTT CAC TTG TAC CCT TTC CTC	3258		
Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu			
1000	1005	1010	
10 AAG GGT TTG ATG GGT CGA CAG AAC CGG ACT CCT ACC ATT GTT GTG GTC	3306		
Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Val Val			
1015	1020	1025	1030
15 TGG TCT CTC TTG GCT TCT ATC TTC TCG TTG TTG TGG GTT AGG ATT	3354		
Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile			
1035	1040	1045	
20 GAT CCC TTC ACT AGC CGA GTC ACT GGC CCG GAC ATT CTG GAA TGT GGA	3402		
Asp Pro Phe Thr Ser Arg Val Thr Gly Pro Asp Ile Leu Glu Cys Gly			
1050	1055	1060	
25 ATC AAC TGT TGAGAACGGA GCAAATATTT ACCTGTTTG AGGGTTAAAA	3451		
Ile Asn Cys			
1065			
30 3511			
25 AAAACACAGA ATTTAAATTAA TTTTCATTG TTTTATTGTG TCACTTTTTT ACTTTTGTG	3511		
TGTGTATCTG TCTGTTCGTT CTTCTGTCTT GGTGTATAA ATTTATGTGT AGAATATATC	3571		
TTACTCTAGT TACTTTGGAA AGTTATAATT AAAGTGAAAG CCA	3614		

(2) INFORMATION FOR SEQ ID NO:10:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1065 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

40

- 144 -

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

5 Met Glu Ser Glu Gly Glu Thr Ala Gly Lys Pro Met Lys Asn Ile Val  
1 5 10 15

Pro Gln Thr Cys Gln Ile Cys Ser Asp Asn Val Gly Lys Thr Val Asp  
20 25 30  
10 Gly Asp Arg Phe Val Ala Cys Asp Ile Cys Ser Phe Pro Val Cys Arg  
35 40 45

Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Asn Gln Ser Cys Pro Gln  
15 50 55 60

Cys Lys Thr Arg Tyr Lys Arg Leu Lys Gly Ser Pro Ala Ile Pro Gly  
65 70 75 80

20 Asp Lys Asp Glu Asp Gly Leu Ala Asp Glu Gly Thr Val Glu Phe Asn  
85 90 95

Tyr Pro Gln Lys Glu Lys Ile Ser Glu Arg Met Leu Gly Trp His Leu  
100 105 110  
25 Thr Arg Gly Lys Gly Glu Met Gly Glu Pro Gln Tyr Asp Lys Glu  
115 120 125

Val Ser His Asn His Leu Pro Arg Leu Thr Ser Arg Gln Asp Thr Ser  
30 130 135 140

Gly Glu Phe Ser Ala Ala Ser Pro Glu Arg Leu Ser Val Ser Ser Thr  
145 150 155 160

35 Ile Ala Gly Gly Lys Arg Leu Pro Tyr Ser Ser Asp Val Asn Gln Ser  
165 170 175

Pro Asn Arg Arg Ile Val Asp Pro Val Gly Leu Gly Asn Val Ala Trp  
180 185 190

40

- 145 -

Lys Glu Arg Val Asp Gly Trp Lys Met Lys Gln Glu Lys Asn Thr Gly  
195 200 205

Pro Val Ser Thr Gln Ala Ala Ser Glu Arg Gly Gly Val Asp Ile Asp  
5 210 215 220

Ala Ser Thr Asp Ile Leu Ala Asp Glu Ala Leu Leu Asn Asp Glu Ala  
225 230 235 240

10 Arg Gln Leu Leu Ser Arg Lys Val Ser Ile Pro Ser Ser Arg Ile Asn  
245 250 255

Pro Tyr Arg Met Val Ile Met Leu Arg Leu Val Ile Leu Cys Leu Phe  
260 265 270

15 Leu His Tyr Arg Ile Thr Asn Pro Val Pro Asn Ala Phe Ala Leu Trp  
275 280 285

Leu Val Ser Val Ile Cys Glu Ile Trp Phe Ala Leu Ser Trp Ile Leu  
20 290 295 300

Asp Gln Phe Pro Lys Trp Phe Pro Val Asn Arg Glu Thr Tyr Leu Asp  
305 310 315 320

25 Arg Leu Ala Leu Arg Tyr Asp Arg Glu Gly Glu Pro Ser Gln Leu Ala  
325 330 335

Ala Val Asp Ile Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro  
340 345 350

30 Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp Tyr Pro  
355 360 365

Val Asp Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu  
35 370 375 380

Ser Phe Glu Ser Leu Ala Glu Thr Ser Glu Phe Ala Arg Lys Trp Val  
385 390 395 400

- 146 -

Pro Phe Cys Lys Lys Tyr Ser Ile Glu Pro Arg Ala Pro Glu Trp Tyr  
405 410 415

Phe Ala Ala Lys Ile Asp Tyr Leu Lys Asp Lys Val Gln Thr Ser Phe  
5 420 425 430

Val Lys Asp Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Ile  
435 440 445

10 Arg Ile Asn Ala Leu Val Ser Lys Ala Leu Lys Cys Pro Glu Glu Gly  
450 455 460

Trp Val Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Thr Gly Asp  
465 470 475 480

15 His Pro Gly Met Ile Gln Val Phe Leu Gly Gln Asn Gly Gly Leu Asp  
485 490 495

Ala Glu Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys  
20 500 505 510

Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu  
515 520 525

25 Val Arg Val Ser Ala Val Leu Thr Asn Gly Pro Phe Ile Leu Asn Leu  
530 535 540

Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Leu Arg Glu Ala Met  
545 550 555 560

30 Cys Phe Leu Met Asp Pro Asn Leu Gly Lys Gln Val Cys Tyr Val Gln  
565 570 575

Phe Pro Gln Arg Phe Asp Gly Ile Asp Lys Asn Asp Arg Tyr Ala Asn  
35 580 585 590

Arg Asn Thr Val Phe Phe Asp Ile Asn Leu Arg Gly Leu Asp Gly Ile  
595 600 605

- 147 -

Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Val Phe Asn Arg Thr Ala  
610 615 620

Leu Tyr Gly Tyr Glu Pro Pro Ile Lys Val Lys His Lys Lys Pro Ser  
5 625 630 635 640

Leu Leu Ser Lys Leu Cys Gly Gly Ser Arg Lys Lys Asn Ser Lys Ala  
645 650 655

10 Lys Lys Glu Ser Asp Lys Lys Ser Gly Arg His Thr Asp Ser Thr  
660 665 670

Val Pro Val Phe Asn Leu Asp Asp Ile Glu Glu Gly Val Glu Gly Ala  
675 680 685

15 Gly Phe Asp Asp Glu Lys Ala Leu Leu Met Ser Gln Met Ser Leu Glu  
690 695 700

Lys Arg Phe Gly Gln Ser Ala Val Phe Val Ala Ser Thr Leu Met Glu  
20 705 710 715 720

Asn Gly Gly Val Pro Pro Ser Ala Thr Pro Glu Asn Phe Leu Lys Glu  
725 730 735

25 Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Ser Asp Trp Gly  
740 745 750

Met Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr  
755 760 765

30 Gly Phe Lys Met His Ala Arg Gly Trp Arg Ser Ile Tyr Cys Met Pro  
770 775 780

Lys Leu Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg  
35 785 790 795 800

Leu Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Leu Phe  
805 810 815

- 148 -

Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Asn Gly Arg Leu Lys Phe  
820 825 830

Leu Glu Arg Phe Ala Tyr Val Asn Thr Thr Ile Tyr Pro Ile Thr Ser  
5 835 840 845

Ile Pro Leu Leu Met Tyr Cys Thr Leu Leu Ala Val Cys Leu Phe Thr  
850 855 860

10 Asn Gln Phe Ile Ile Pro Gln Ile Ser Asn Ile Ala Ser Ile Trp Phe  
865 870 875 880

Leu Ser Leu Phe Leu Ser Ile Phe Ala Thr Gly Ile Leu Glu Met Arg  
885 890 895

15 Trp Ser Gly Val Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe Trp  
900 905 910

Val Ile Gly Gly Val Ser Ala His Leu Phe Ala Val Phe Gln Gly Ile  
20 915 920 925

Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys  
930 935 940

25 Ala Ser Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Leu Phe Lys Trp  
945 950 955 960

Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Ile Val Asn Leu Val  
965 970 975

30 Gly Val Val Ala Gly Val Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser  
980 985 990

Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile Val  
35 995 1000 1005

His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg Thr  
1010 1015 1020

- 149 -

Pro Thr Ile Val Val Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser  
1025 1030 1035 1040

Leu Leu Trp Val Arg Ile Asp Pro Phe Thr Ser Arg Val Thr Gly Pro  
5 1045 1050 1055

Asp Ile Leu Glu Cys Gly Ile Asn Cys  
1060 1065

10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 3673 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Arabidopsis thaliana*  
(B) STRAIN: Columbia  
(C) INDIVIDUAL ISOLATE: rsw1 mutant

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(ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 71..3313

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATCGGCTA CGAATTTCCC AATTTTGAAT TTTGTGAATC TCTCTCTTTC TCTGTGTGTC

60

- 150 -

GGTGGCTGCG ATG GAG GCC AGT GCC GGC TTG GTT GCT GGA TCC TAC CGG			109
Met Glu Ala Ser Ala Gly Leu Val Ala Gly Ser Tyr Arg			
1	5	10	
5 AGA AAC GAG CTC GTT CGG ATC CGA CAT GAA TCT GAT GGC GGG ACC AAA			157
Arg Asn Glu Leu Val Arg Ile Arg His Glu Ser Asp Gly Gly Thr Lys			
15	20	25	
CCT TTG AAG AAT ATG AAT GGC CAG ATA TGT CAG ATC TGT GGT GAT GAT			205
10 Pro Leu Lys Asn Met Asn Gly Gln Ile Cys Gln Ile Cys Gly Asp Asp			
30	35	40	45
GTT GGA CTC GCT GAA ACT GGA GAT GTC TTT GTC GCG TGT AAT GAA TGT			253
Val Gly Leu Ala Glu Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys			
15	50	55	60
GCC TTC CCT GTG TGT CGG CCT TGC TAT GAG TAC GAG AGG AAA GAT GGA			301
Ala Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly			
65	70	75	
20			
ACT CAG TGT TGC CCT CAA TGC AAG ACT AGA TTC AGA CGA CAC AGG GGG			349
Thr Gln Cys Cys Pro Gln Cys Lys Thr Arg Phe Arg Arg His Arg Gly			
80	85	90	
25 AGT CCT CGT GTT GAA GGA GAT GAA GAT GAG GAT GAT GTT GAT GAT ATC			397
Ser Pro Arg Val Glu Gly Asp Glu Asp Glu Asp Asp Val Asp Asp Ile			
95	100	105	
GAG AAT GAG TTC AAT TAC GCC CAG GGA GCT AAC AAG GCG AGA CAC CAA			445
30 Glu Asn Glu Phe Asn Tyr Ala Gln Gly Ala Asn Lys Ala Arg His Gln			
110	115	120	125
CGC CAT GGC GAA GAG TTT TCT TCT TCC TCT AGA CAT GAA TCT CAA CCA			493
Arg His Gly Glu Glu Phe Ser Ser Ser Ser Arg His Glu Ser Gln Pro			
35	130	135	140
ATT CCT CTT CTC ACC CAT GGC CAT ACG GTT TCT GGA GAG ATT CGC ACG			541
Ile Pro Leu Leu Thr His Gly His Thr Val Ser Gly Glu Ile Arg Thr			
145	150	155	
40			

- 151 -

CCT GAT ACA CAA TCT GTG CGA ACT ACA TCA GGT CCT TTG GGT CCT TCT		589
Pro Asp Thr Gln Ser Val Arg Thr Thr Ser Gly Pro Leu Gly Pro Ser		
160	165	170
 5 GAC AGG AAT GCT ATT TCA TCT CCA TAT ATT GAT CCA CGG CAA CCT GTC		637
Asp Arg Asn Ala Ile Ser Ser Pro Tyr Ile Asp Pro Arg Gln Pro Val		
175	180	185
 CCT GTA AGA ATC GTG GAC CCG TCA AAA GAC TTG AAC TCT TAT GGG CTT		685
10 Pro Val Arg Ile Val Asp Pro Ser Lys Asp Leu Asn Ser Tyr Gly Leu		
190	195	200
 15 GGT AAT GTT GAC TGG AAA GAA AGA GTT GAA GGC TGG AAG CTG AAG CAG		733
Gly Asn Val Asp Trp Lys Glu Arg Val Glu Gly Trp Lys Leu Lys Gln		
210	215	220
 GAG AAA AAT ATG TTA CAG ATG ACT GGT AAA TAC CAT GAA GGG AAA GGA		781
Glu Lys Asn Met Leu Gln Met Thr Gly Lys Tyr His Glu Gly Lys Gly		
225	230	235
 20 GGA GAA ATT GAA GGG ACT GGT TCC AAT GGC GAA GAA CTC CAA ATG GCT		829
Gly Glu Ile Glu Gly Thr Gly Ser Asn Gly Glu Glu Leu Gln Met Ala		
240	245	250
 25 GAT GAT ACA CGT CTT CCT ATG AGT CGT GTG GTG CCT ATC CCA TCT TCT		877
Asp Asp Thr Arg Leu Pro Met Ser Arg Val Val Pro Ile Pro Ser Ser		
255	260	265
 CGC CTA ACC CCT TAT CGG GTT GTG ATT ATT CTC CGG CTT ATC ATC TTG		925
30 Arg Leu Thr Pro Tyr Arg Val Val Ile Ile Leu Arg Leu Ile Ile Leu		
270	275	280
 35 TGT TTC TTC TTG CAA TAT CGT ACA ACT CAC CCT GTG AAA AAT GCA TAT		973
Cys Phe Phe Leu Gln Tyr Arg Thr Thr His Pro Val Lys Asn Ala Tyr		
290	295	300
 CCT TTG TGG TTG ACC TCG GTT ATC TGT GAG ATC TGG TTT GCA TTT TCT		1021
Pro Leu Trp Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Phe Ser		
305	310	315
 40		

- 152 -

TGG CTT CTT GAT CAG TTT CCC AAA TGG TAC CCC ATT AAC AGG GAG ACT	1069		
Trp Leu Leu Asp Gln Phe Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr			
320	325	330	
 5 TAT CTT GAC CGT CTC GCT ATA AGA TAT GAT CGA GAC GGT GAA CCA TCA	1117		
Tyr Leu Asp Arg Leu Ala Ile Arg Tyr Asp Arg Asp Gly Glu Pro Ser			
335	340	345	
 10 CAG CTC CCT GTT GAT GTG TTT GTT AGT ACA GTG GAC CCA TTG AAA	1165		
Gln Leu Val Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys			
350	355	360	365
 15 GAG CCT CCC CTT GTT ACA GCA AAC ACA GTT CTC TCG ATT CTT TCT GTG	1213		
Glu Pro Pro Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val			
370	375	380	
 20 GAC TAC CCG GTA GAT AAA GTA GCC TGT TAT GTT TCA GAT GAT GGT TCA	1261		
Asp Tyr Pro Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ser			
385	390	395	
 25 GCT ATG CTT ACC TTT GAA TCC CTT TCT GAA ACC GCT GAG TTT GCA AAG	1309		
Ala Met Leu Thr Phe Glu Ser Leu Ser Glu Thr Ala Glu Phe Ala Lys			
400	405	410	
 30 GAA TTC TAT TTT GCC CAG AAG ATA GAT TAC TTG AAG GAC AAG ATC CAA	1357		
Lys Trp Val Pro Phe Cys Lys Phe Asn Ile Glu Pro Arg Ala Pro			
415	420	425	
 35 GCG TCT TTT GTT AAA GAG CGA CGA GCT ATG AAG AGA GAG TAT GAA GAG	1405		
Pro Ser Phe Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu			
430	435	440	445
 40 TTT AAA GTG AGG ATA AAT GCT CTT GTT GCC AAA GCA CAG AAA ATC CCT	1453		
Phe Lys Val Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Ile Pro			
465	470	475	

- 153 -

GAA GAA GGC TGG ACA ATG CAG GAT GGT ACT CCC TGG CCT GGT AAC AAC			1549
Glu Glu Gly Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn			
480	485	490	
 5 ACT AGA GAT CAT CCT GGA ATG ATA CAG GTG TTC TTA GGC CAT AGT GGG			1597
Thr Arg Asp His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly			
495	500	505	
 GGT CTG GAT ACC GAT GGA AAT GAG CTG CCT AGA CTC ATC TAT GTT TCT			1645
10 Gly Leu Asp Thr Asp Gly Asn Glu Leu Pro Arg Leu Tyr Val Ser			
510	515	520	525
 CGT GAA AAG CGG CCT GGA TTT CAA CAC CAC AAA AAG GCT GGA GCT ATG			1693
Arg Glu Lys Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met			
15	530	535	540
 AAT GCA TTG ATC CGT GTA TCT GTT CTT ACC AAT GGA GCA TAT CTT			1741
Asn Ala Leu Ile Arg Val Ser Val Val Leu Thr Asn Gly Ala Tyr Leu			
545	550	555	
20 TTG AAC GTG GAT TGT GAT CAT TAC TTT AAT AAC AGT AAG GCT ATT AAA			1789
Leu Asn Val Asp Cys Asp His Tyr Phe Asn Asn Ser Lys Ala Ile Lys			
560	565	570	
 25 GAA GCT ATG TGT TTC ATG ATG GAC CCG GCT ATT GGA AAG AAG TGC TGC			1837
Glu Ala Met Cys Phe Met Met Asp Pro Ala Ile Gly Lys Lys Cys Cys			
575	580	585	
 TAT GTC CAG TTC CCT CAA CGT TTT GAC GGT ATT GAT TTG CAC GAT CGA			1885
30 Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Leu His Asp Arg			
590	595	600	605
 TAT GCC AAC AGG AAT ATA GTC TTT TTC GAT ATT AAC ATG AAG GGG TTG			1933
Tyr Ala Asn Arg Asn Ile Val Phe Phe Asp Ile Asn Met Lys Gly Leu			
35	610	615	620
 GAT GGT ATC CAG GGT CCA GTA TAT GTG GGT ACT GGT TGT TGT TTT AAT			1981
Asp Gly Ile Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Cys Phe Asn			
625	630	635	

- 154 -

AGG CAG GCT CTA TAT GGG TAT GAT CCT GTT TTG ACG GAA GAA GAT TTA			2029
Arg Gln Ala Leu Tyr Gly Tyr Asp Pro Val Leu Thr Glu Glu Asp Leu			
640	645	650	
 5 GAA CCA AAT ATT ATT GTC AAG AGC TGT TGC GGG TCA AGG AAG AAA GGT			2077
Glu Pro Asn Ile Ile Val Lys Ser Cys Cys Gly Ser Arg Lys Lys Gly			
655	660	665	
 AAA AGT AGC AAG AAG TAT AAC TAC GAA AAG AGG AGA GGC ATC AAC AGA			2125
10 Lys Ser Ser Lys Lys Tyr Asn Tyr Glu Lys Arg Arg Gly Ile Asn Arg			
670	675	680	685
 AGT GAC TCC AAT GCT CCA CTT TTC AAT ATG GAG GAC ATC GAT GAG GGT			2173
Ser Asp Ser Asn Ala Pro Leu Phe Asn Met Glu Asp Ile Asp Glu Gly			
15	690	695	700
 TTT GAA GGT TAT GAT GAT GAG AGG TCT ATT CTA ATG TCC CAG AGG AGT			2221
Phe Glu Gly Tyr Asp Asp Glu Arg Ser Ile Leu Met Ser Gln Arg Ser			
705	710	715	
20 GTA GAG AAG CGT TTT GGT CAG TCG CCG GTA TTT ATT GCG GCA ACC TTC			2269
Val Glu Lys Arg Phe Gly Gln Ser Pro Val Phe Ile Ala Ala Thr Phe			
720	725	730	
 25 ATG GAA CAA GGC GGC ATT CCA CCA ACA ACC AAT CCC GCT ACT CTT CTG			2317
Met Glu Gln Gly Gly Ile Pro Pro Thr Thr Asn Pro Ala Thr Leu Leu			
735	740	745	
 AAG GAG GCT ATT CAT GTT ATA AGC TGT GGT TAC GAA GAC AAG ACT GAA			2365
30 Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu			
750	755	760	765
 TGG GGC AAA GAG ATT GGT TGG ATC TAT GGT TCC GTG ACG GAA GAT ATT			2413
Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile			
35	770	775	780
 CTT ACT GGG TTC AAG ATG CAT GCC CGG GGT TGG ATA TCG ATC TAC TGC			2461
Leu Thr Gly Phe Lys Met His Ala Arg Gly Trp Ile Ser Ile Tyr Cys			
785	790	795	

- 155 -

AAT CCT CCA CGC CCT GCG TTC AAG GGA TCT GCA CCA ATC AAT CTT TCT Asn Pro Pro Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser	2509
800                    805                    810	
 5 GAT CGT TTG AAC CAA GTT CTT CGA TGG GCT TTG GGA TCT ATC GAG ATT Asp Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Ile Glu Ile	2557
815                    820                    825	
 10 CTT CTT AGC AGA CAT TGT CCT ATC TGG TAT GGT TAC CAT GGA AGG TTG Leu Leu Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr His Gly Arg Leu	2605
830                    835                    840                    845	
 15 AGA CTT TTG GAG AGG ATC GCT TAT ATC AAC ACC ATC GTC TAT CCT ATT Arg Leu Leu Glu Arg Ile Ala Tyr Ile Asn Thr Ile Val Tyr Pro Ile	2653
850                    855                    860	
 20 ACA TCC ATC CCT CTT ATT GCG TAT TGT ATT CTT CCC GCT TTT TGT CTC Thr Ser Ile Pro Leu Ile Ala Tyr Cys Ile Leu Pro Ala Phe Cys Leu	2701
865                    870                    875	
 25 TGG TTC ATT CTA CTC TTC ATC TCA ATT GCT GTG ACT GGA ATC CTG GAG Trp Phe Ile Leu Leu Phe Ile Ser Ile Ala Val Thr Gly Ile Leu Glu	2749
880                    885                    890	
 30 CTG AGA TGG AGC GGT GTG AGC ATT GAG GAT TGG TGG AGG AAC GAG CAG Leu Arg Trp Ser Gly Val Ser Ile Glu Asp Trp Trp Arg Asn Glu Gln	2797
895                    900                    905	
 35 TTC TGG GTC ATT GGT GGC ACA TCC GCC CAT CTT TTT GCT GTC TTC CAA Phe Trp Val Ile Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln	2845
910                    915                    920                    925	
 40 GGT CTA CTT AAG GTT CTT GCT GGT ATC GAC ACC AAC TTC ACC GTT ACA Gly Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr	2893
930                    935                    940	
 45 945                    950                    955	2941

- 156 -

TCT AAA GCC ACA GAC GAA GAT GGG GAT TTT GCA GAA CTC TAC ATC TTC		2989
Ser Lys Ala Thr Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Ile Phe		
960	965	970
 5 AAA TGG ACA GCT CTT CTC ATT CCA CCA ACC ACC GTC CTA CTT GTG AAC		3037
Lys Trp Thr Ala Leu Leu Ile Pro Pro Thr Thr Val Leu Leu Val Asn		
975	980	985
 10 CTC ATA GGC ATT GTG GCT GGT GTC TCT TAT GCT GTA AAC AGT GGC TAC		3085
Leu Ile Gly Ile Val Ala Gly Val Ser Tyr Ala Val Asn Ser Gly Tyr		
990	995	1000
 15 CAG TCG TGG GGT CCG CTT TTC GGG AAG CTC TTC GCC TTA TGG GTT		3133
Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Leu Trp Val		
1010	1015	1020
 20 ATT GCC CAT CTC TAC CCT TTC TTG AAA GGT CTG TTG GGA AGA CAA AAC		3181
Ile Ala His Leu Tyr Pro Phe Leu Lys Gly Leu Leu Gly Arg Gln Asn		
1025	1030	1035
 25 CGA ACA CCA ACC ATC GTC ATT GTC TGG TCT GTT CTT CTC GCC TCC ATC		3229
Arg Thr Pro Thr Ile Val Ile Val Trp Ser Val Leu Leu Ala Ser Ile		
1040	1045	1050
 30 TTC TCG TTG CTT TGG GTC AGG ATC AAT CCC TTT GTG GAC GCC AAT CCC		3277
Phe Ser Leu Leu Trp Val Arg Ile Asn Pro Phe Val Asp Ala Asn Pro		
1055	1060	1065
 35 AAT GCC AAC AAC TTC AAT GGC AAA GGA GGT GTC TTT TAGACCCTAT		3323
Asn Ala Asn Asn Phe Asn Gly Lys Gly Gly Val Phe		
1070	1075	1080
 40 TTATATACTT GTGTGTGCAT ATATCAAAAA CGCGCAATGG GAATTCCAAA TCATCTAAC		3383
CCATCAAACC CCAGTGAACC GGGCAGTTAA GGTGATTCCA TGTCCAAGAT TAGCTTCTC		3443
CGAGTAGCCA GAGAAGGTGA AATTGTTCGT AACACTATTG TAATGATTTT CCAGTGGGA		3503
AGAAGATGTG GACCCAAATG ATACATAGTC TACAAAAAGA ATTTGTTATT CTTCTTATA		3563

- 157 -

TTTATTTAT TTAAAGCTTG TTAGACTCAC ACTTATGTAA TGTTGGAAC TGGTGTCTA 3623

AAAAGGGATT GGAGTTTCT TTTATCTAA GAATCTGAAG TTTATATGCT 3673

5

(2) INFORMATION FOR SEQ ID NO:12:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1081 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Glu Ala Ser Ala Gly Leu Val Ala Gly Ser Tyr Arg Arg Asn Glu

20 1 5 10 15

Leu Val Arg Ile Arg His Glu Ser Asp Gly Gly Thr Lys Pro Leu Lys

20 25 30

25 Asn Met Asn Gly Gln Ile Cys Gln Ile Cys Gly Asp Asp Val Gly Leu

35 40 45

Ala Glu Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala Phe Pro

50 55 60

30 Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln Cys

65 70 75 80

Cys Pro Gln Cys Lys Thr Arg Phe Arg Arg His Arg Gly Ser Pro Arg

35 85 90 95

Val Glu Gly Asp Glu Asp Asp Val Asp Asp Ile Glu Asn Glu

100 105 110

- 158 -

Phe Asn Tyr Ala Gln Gly Ala Asn Lys Ala Arg His Gln Arg His Gly  
115 120 125

Glu Glu Phe Ser Ser Ser Arg His Glu Ser Gln Pro Ile Pro Leu  
5 130 135 140

Leu Thr His Gly His Thr Val Ser Gly Glu Ile Arg Thr Pro Asp Thr  
145 150 155 160

10 Gln Ser Val Arg Thr Thr Ser Gly Pro Leu Gly Pro Ser Asp Arg Asn  
165 170 175

Ala Ile Ser Ser Pro Tyr Ile Asp Pro Arg Gln Pro Val Pro Val Arg  
180 185 190

15 Ile Val Asp Pro Ser Lys Asp Leu Asn Ser Tyr Gly Leu Gly Asn Val  
195 200 205

Asp Trp Lys Glu Arg Val Glu Gly Trp Lys Leu Lys Gln Glu Lys Asn  
20 210 215 220

Met Leu Gln Met Thr Gly Lys Tyr His Glu Gly Lys Gly Glu Ile  
225 230 235 240

25 Glu Gly Thr Gly Ser Asn Gly Glu Glu Leu Gln Met Ala Asp Asp Thr  
245 250 255

Arg Leu Pro Met Ser Arg Val Val Pro Ile Pro Ser Ser Arg Leu Thr  
260 265 270

30 Pro Tyr Arg Val Val Ile Ile Leu Arg Leu Ile Ile Leu Cys Phe Phe  
275 280 285

Leu Gln Tyr Arg Thr Thr His Pro Val Lys Asn Ala Tyr Pro Leu Trp  
35 290 295 300

Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Phe Ser Trp Leu Leu  
305 310 315 320

- 159 -

Asp Gln Phe Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp  
325 330 335

Arg Leu Ala Ile Arg Tyr Asp Arg Asp Gly Glu Pro Ser Gln Leu Val  
5 340 345 350

Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro  
355 360 365

10 Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro  
370 375 380

Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ser Ala Met Leu  
385 390 395 400

15 Thr Phe Glu Ser Leu Ser Glu Thr Ala Glu Phe Ala Lys Lys Trp Val  
405 410 415

Pro Phe Cys Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr  
20 420 425 430

Phe Ala Gln Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser Phe  
435 440 445

25 Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val  
450 455 460

Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Ile Pro Glu Glu Gly  
465 470 475 480

30 Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Thr Arg Asp  
485 490 495

His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp  
35 500 505 510

Thr Asp Gly Asn Glu Leu Pro Arg Leu Ile Tyr Val Ser Arg Glu Lys  
515 520 525

- 160 -

Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu  
530 535 540

Ile Arg Val Ser Val Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val  
5 545 550 555 560

Asp Cys Asp His Tyr Phe Asn Asn Ser Lys Ala Ile Lys Glu Ala Met  
565 570 575

10 Cys Phe Met Met Asp Pro Ala Ile Gly Lys Lys Cys Cys Tyr Val Gln  
580 585 590

Phe Pro Gln Arg Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn  
595 600 605

15 Arg Asn Ile Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile  
610 615 620

Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala  
20 625 630 635 640

Leu Tyr Gly Tyr Asp Pro Val Leu Thr Glu Glu Asp Leu Glu Pro Asn  
645 650 655

25 Ile Ile Val Lys Ser Cys Cys Gly Ser Arg Lys Lys Gly Lys Ser Ser  
660 665 670

Lys Lys Tyr Asn Tyr Glu Lys Arg Arg Gly Ile Asn Arg Ser Asp Ser  
675 680 685

30 Asn Ala Pro Leu Phe Asn Met Glu Asp Ile Asp Glu Gly Phe Glu Gly  
690 695 700

Tyr Asp Asp Glu Arg Ser Ile Leu Met Ser Gln Arg Ser Val Glu Lys  
35 705 710 715 720

Arg Phe Gly Gln Ser Pro Val Phe Ile Ala Ala Thr Phe Met Glu Gln  
725 730 735

- 161 -

Gly Gly Ile Pro Pro Thr Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala  
740 745 750

Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys  
5 755 760 765

Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly  
770 775 780

10 Phe Lys Met His Ala Arg Gly Trp Ile Ser Ile Tyr Cys Asn Pro Pro  
785 790 795 800

Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu  
805 810 815

15 Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Ile Glu Ile Leu Leu Ser  
820 825 830

Arg His Cys Pro Ile Trp Tyr Gly Tyr His Gly Arg Leu Arg Leu Leu  
20 835 840 845

Glu Arg Ile Ala Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile  
850 855 860

25 Pro Leu Ile Ala Tyr Cys Ile Leu Pro Ala Phe Cys Leu Ile Thr Asp  
865 870 875 880

Arg Phe Ile Ile Pro Glu Ile Ser Asn Tyr Ala Ser Ile Trp Phe Ile  
885 890 895

30 Leu Leu Phe Ile Ser Ile Ala Val Thr Gly Ile Leu Glu Leu Arg Trp  
900 905 910

Ser Gly Val Ser Ile Glu Asp Trp Trp Arg Asn Glu Gln Phe Trp Val  
35 915 920 925

Ile Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu  
930 935 940

- 162 -

Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala  
945 950 955 960

5 Thr Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Ile Phe Lys Trp Thr  
965 970 975

Ala Leu Leu Ile Pro Pro Thr Thr Val Leu Leu Val Asn Leu Ile Gly  
980 985 990

10 Ile Val Ala Gly Val Ser Tyr Ala Val Asn Ser Gly Tyr Gln Ser Trp  
995 1000 1005

Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Leu Trp Val Ile Ala His  
1010 1015 1020

15 Leu Tyr Pro Phe Leu Lys Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro  
1025 1030 1035 1040

Thr Ile Val Ile Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu  
20 1045 1050 1055

Leu Trp Val Arg Ile Asn Pro Phe Val Asp Ala Asn Pro Asn Ala Asn  
1060 1065 1070

25 Asn Phe Asn Gly Lys Gly Val Phe  
1075 1080

30 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1741 base pairs  
(B) TYPE: nucleic acid  
35 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

40 (iii) HYPOTHETICAL: NO

- 163 -

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Oryza sativa

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(vii) IMMEDIATE SOURCE:

(B) CLONE: S0542

(ix) FEATURE:

10

(A) NAME/KEY: CDS

(B) LOCATION: 101..1741

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

15

GTGCGGCCGC CGCGCATCTA GGCTTGCCGC GCGCGCCGG ATCTGCGAGC TCGCTAGCCG	60
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TTTCTCGCTG TGAGTGGAGG AGGAGGAGGA AGGGAGGAGG ATG GCG GCG AAC GCG Met Ala Ala Asn Ala	115
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20

1	5
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GGG ATG GTG GCG GGA TCC CGC AAC CGG AAC GAG TTC GTC ATG ATC CGC Gly Met Val Ala Gly Ser Arg Asn Arg Asn Glu Phe Val Met Ile Arg	163
--	-----

10	15	20
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25

CCC GAC GGC GAC GCG CCA CCG CCG GCT AAG CCA GGG AAG AGT GTG AAT Pro Asp Gly Asp Ala Pro Pro Pro Ala Lys Pro Gly Lys Ser Val Asn	211
--	-----

25	30	35
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30

GGT CAG GTC TGC CAG ATT TGT GGC GAC ACT GTT GGC GTC TCG GCC ACC Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val Gly Val Ser Ala Thr	259
--	-----

40	45	50
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35

GGC GAC GTC TTT GTT GCC TGC AAT GAG TGC GCC TTC CCG GTC TGC CGC Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala Phe Pro Val Cys Arg	307
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55	60	65
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40

CCT TGC TAC GAG TAC GAA CGC AAG GAA GGG AAC CAG TGC TGC CCC CAG Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn Gln Cys Cys Pro Gln	355
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70	75	80
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	85
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- 164 -

TGC AAG ACT AGA TAC AAG AGG CAC AAA GGT TGC CCT AGA GTT CAG GGC			403
Cys Lys Thr Arg Tyr Lys Arg His Lys Gly Cys Pro Arg Val Gln Gly			
90	95	100	
5 GAT GAG GAA GAA GAA GAT GTT GAT GAC CTG GAC AAT GAA TTC CAT TAT			451
Asp Glu Glu Glu Asp Val Asp Asp Leu Asp Asn Glu Phe His Tyr			
105	110	115	
AAG CAT GGC AAT GGC AAA GGT CCA GAG TGG CAG ATA CAG AGA CAG GGG			499
Lys His Gly Asn Gly Lys Gly Pro Glu Trp Gln Ile Gln Arg Gln Gly			
120	125	130	
GAA GAT GTT GAC CTG TCT TCA TCT TCT CGC CAC GAA CAA CAT CGG ATT			547
Glu Asp Val Asp Leu Ser Ser Ser Arg His Glu Gln His Arg Ile			
15 135	140	145	
CCC CGT CTG ACA AGT GGG CAA CAG ATC TCA GGA GAG ATC CCT GAT GCT			595
Pro Arg Leu Thr Ser Gly Gln Gln Ile Ser Gly Glu Ile Pro Asp Ala			
150	155	160	165
20			
TCC CCC GAT CGC CAT TCT ATC CGC AGC GGA ACA TCA AGC TAT GTT GAT			643
Ser Pro Asp Arg His Ser Ile Arg Ser Gly Thr Ser Ser Tyr Val Asp			
170	175	180	
25			
CCA AGT GTT CCA GTT CCT GTG AGG ATT GTG GAC CCC TCC AAG GAC TTG			691
Pro Ser Val Pro Val Pro Val Arg Ile Val Asp Pro Ser Lys Asp Leu			
185	190	195	
AAT TCC TAT GGG ATT AAC AGT GTT GAC TGG CAA GAA AGA GTT GCC AGC			739
30 Asn Ser Tyr Gly Ile Asn Ser Val Asp Trp Gln Glu Arg Val Ala Ser			
200	205	210	
TGG AGG AAC AAG CAG GAC AAA AAT ATG ATG CAG GTA GCT AAT AAA TAT			787
Trp Arg Asn Lys Gln Asp Lys Asn Met Met Gln Val Ala Asn Lys Tyr			
35 215	220	225	
CCA GAG GCA AGA GGG GGA GAC ATG GAA GGG ACT GGT TCA AAT GGT GAA			835
Pro Glu Ala Arg Gly Gly Asp Met Glu Gly Thr Gly Ser Asn Gly Glu			
230	235	240	245

- 165 -

GAT ATC CAA ATG GTT GAT GAT GCA CGT CTA CCT CTG AGC CGC ATA GTG			883
Asp Ile Gln Met Val Asp Asp Ala Arg Leu Pro Leu Ser Arg Ile Val			
250	255	260	
 5 CCT ATC CCT TCA AAC CAG CTC AAC CTT TAC CGG ATT GTT ATC ATT CTC			931
Pro Ile Pro Ser Asn Gln Leu Asn Leu Tyr Arg Ile Val Ile Ile Leu			
265	270	275	
 CGT CTT ATC ATC CTG ATG TTC TTC CAA TAT CGT GTC ACT CAT CCA			979
10 Arg Leu Ile Ile Leu Met Phe Phe Phe Gln Tyr Arg Val Thr His Pro			
280	285	290	
 GTG CGG GAT GCT TAT GGA TTG TGG CTA GTA TCT GTT ATC TGT GAA ATT			1027
Val Arg Asp Ala Tyr Gly Leu Trp Leu Val Ser Val Ile Cys Glu Ile			
15 295	300	305	
 TGG TTG CCC TTA TCC TGG CTC CTA GAT CAA TTC CCA AAG TGG TAC CCG			1075
Trp Leu Pro Leu Ser Trp Leu Leu Asp Gln Phe Pro Lys Trp Tyr Pro			
310	315	320	325
 20 ATA AAC CGT GAA ACA TAC CTT GAC AGG CTT GCA TTG AGA TAT GAT AGG			1123
Ile Asn Arg Glu Thr Tyr Leu Asp Arg Leu Ala Leu Arg Tyr Asp Arg			
330	335	340	
 25 GAG GGA GAG CCA TCA CAG CTT GCT CCC ATT GAT GTC TTT GTC AGT ACG			1171
Glu Gly Glu Pro Ser Gln Leu Ala Pro Ile Asp Val Phe Val Ser Thr			
345	350	355	
 GTG GAT CCA CTA AAG GAA CCT CCT CTG ATC ACA GCA AAC ACT GTT TTG			1219
30 Val Asp Pro Leu Lys Glu Pro Pro Leu Ile Thr Ala Asn Thr Val Leu			
360	365	370	
 TCC ATT CTG GCT GTG GAT TAC CCT GTT GAC AAA GTG TCA TGC TAT GTT			1267
Ser Ile Leu Ala Val Asp Tyr Pro Val Asp Lys Val Ser Cys Tyr Val			
35 375	380	385	
 TCT GAC GAT GGT TCA GCT ATG TTA ACT TTT GAG GCT CTG TCA GAA ACT			1315
Ser Asp Asp Gly Ser Ala Met Leu Thr Phe Glu Ala Leu Ser Glu Thr			
390	395	400	405
 40			

- 166 -

GCA GAA TTT GCT AGG AAG TGG GTT CCG TTT TGC AAG AAG CAC AAT ATT			1363
Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys Lys Lys His Asn Ile			
410	415	420	
 5 GAA CCA CGA GCT CCA GAG TTT TAC TTT GCT CAA AAA ATA GAT TAC CTG			1411
Glu Pro Arg Ala Pro Glu Phe Tyr Phe Ala Gln Lys Ile Asp Tyr Leu			
425	430	435	
 AAG GAC AAA ATC CAA CCT TCC TTT GTT AAA GAA AGG CGG GCA ATG AAG			1459
10 Lys Asp Lys Ile Gln Pro Ser Phe Val Lys Glu Arg Arg Ala Met Lys			
440	445	450	
 AGA GAG TAT GAA GAA TTC AAG GTA CGG ATC AAT GCT CTT GTT GCG AAG			1507
Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Leu Val Ala Lys			
15 455	460	465	
 GCA CAA AAA GTA CCT GAA GAG GGG TGG ACC ATG GCT GAT GGC ACT GCT			1555
Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met Ala Asp Gly Thr Ala			
470	475	480	485
 20 TGG CCT GGG AAT AAC CCA AGG GAT CAC CCT GGC ATG ATT CAG GTG TTC			1603
Trp Pro Gly Asn Asn Pro Arg Asp His Pro Gly Met Ile Gln Val Phe			
490	495	500	
 25 TTG GGG CAC AGT GGT GGG CTT GAC ACT GAT GGT AAC GAG TTG CCA CGG			1651
Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly Asn Glu Leu Pro Arg			
505	510	515	
 30 CTT GTC TAC GTC TCT CGT GAA AAG AGG CCA GGA TTC CAG CAT CAC AAG			1699
Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe Gln His His Lys			
520	525	530	
 AAG GCT GGT GCA ATG AAT GCA TTG ATT CGT GTA TCT GCT GTG			1741
Lys Ala Gly Ala Met Asn Ala Leu Ile Arg Val Ser Ala Val			
35 535	540	545	

- 167 -

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 547 amino acids

5 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ala Ala Asn Ala Gly Met Val Ala Gly Ser Arg Asn Arg Asn Glu

1 5 10 15

15 Phe Val Met Ile Arg Pro Asp Gly Asp Ala Pro Pro Pro Ala Lys Pro

20 25 30

Gly Lys Ser Val Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val

35 40 45

20

Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala

50 55 60

Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn

25 65 70 75 80

Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg His Lys Gly Cys

85 90 95

30 Pro Arg Val Gln Gly Asp Glu Glu Glu Asp Val Asp Asp Leu Asp

100 105 110

Asn Glu Phe His Tyr Lys His Gly Asn Gly Lys Gly Pro Glu Trp Gln

115 120 125

35

Ile Gln Arg Gln Gly Glu Asp Val Asp Leu Ser Ser Ser Ser Arg His

130 135 140

Glu Gln His Arg Ile Pro Arg Leu Thr Ser Gly Gln Gln Ile Ser Gly

40 145 150 155 160

- 168 -

Glu Ile Pro Asp Ala Ser Pro Asp Arg His Ser Ile Arg Ser Gly Thr  
165 170 175

Ser Ser Tyr Val Asp Pro Ser Val Pro Val Pro Val Arg Ile Val Asp  
5 180 185 190

Pro Ser Lys Asp Leu Asn Ser Tyr Gly Ile Asn Ser Val Asp Trp Gln  
195 200 205

10 Glu Arg Val Ala Ser Trp Arg Asn Lys Gln Asp Lys Asn Met Met Gln  
210 215 220

Val Ala Asn Lys Tyr Pro Glu Ala Arg Gly Gly Asp Met Glu Gly Thr  
225 230 235 240

15 Gly Ser Asn Gly Glu Asp Ile Gln Met Val Asp Asp Ala Arg Leu Pro  
245 250 255

Leu Ser Arg Ile Val Pro Ile Pro Ser Asn Gln Leu Asn Leu Tyr Arg  
20 260 265 270

Ile Val Ile Ile Leu Arg Leu Ile Ile Leu Met Phe Phe Phe Gln Tyr  
275 280 285

25 Arg Val Thr His Pro Val Arg Asp Ala Tyr Gly Leu Trp Leu Val Ser  
290 295 300

Val Ile Cys Glu Ile Trp Leu Pro Leu Ser Trp Leu Leu Asp Gln Phe  
305 310 315 320

30 Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp Arg Leu Ala  
325 330 335

Leu Arg Tyr Asp Arg Glu Gly Glu Pro Ser Gln Leu Ala Pro Ile Asp  
35 340 345 350

Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Ile Thr  
355 360 365

- 169 -

Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp Tyr Pro Val Asp Lys  
370                   375                   380

5 Val Ser Cys Tyr Val Ser Asp Asp Gly Ser Ala Met Leu Thr Phe Glu  
385                   390                   395                   400

Ala Leu Ser Glu Thr Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys  
405                   410                   415

10

Lys Lys His Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr Phe Ala Gln  
420                   425                   430

15

Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser Phe Val Lys Glu  
435                   440                   445

Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn  
450                   455                   460

20

Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met  
465                   470                   475                   480

Ala Asp Gly Thr Ala Trp Pro Gly Asn Asn Pro Arg Asp His Pro Gly  
485                   490                   495

25

Met Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly  
500                   505                   510

30

Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly  
515                   520                   525

Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu Ile Arg Val  
530                   535                   540

35 Ser Ala Val

545

40

- 170 -

**CLAIMS:**

1. An isolated nucleic acid molecule which encodes a polypeptide of the cellulose biosynthetic pathway or a homologue, analogue or derivative thereof or a complementary sequence thereto, wherein said polypeptide is capable of producing cellulose and/or  $\beta$ -1,4-glucan and/or an intermediate between cellulose and a  $\beta$ -1,4-glucan polymer.
2. The isolated nucleic acid molecule according to claim 1 wherein the polypeptide is cellulose synthase or a catalytic subunit thereof.
- 10 3. The isolated nucleic acid molecule according to claim 1 or 2, derived from a prokaryote.
4. The isolated nucleic acid molecule according to claim 3, wherein the prokaryote is a bacterium other than *Agrobacterium tumefaciens*, *Acetobacter pasteurianus* or *Acetobacter xylinum*.
- 15 5. The isolated nucleic acid molecule according to claim 1 or 2, derived from a eukaryote.
- 20 6. The isolated nucleic acid molecule according to claim 5, wherein the eukaryote is a plant or fungus.
7. The isolated nucleic acid molecule according to claim 6, wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* 25 (rice), wheat, barley, maize, *Brassica* spp., *Eucalyptus* spp., hemp, jute, flax, *Pinus* spp., *Populus* spp., and *Picea* spp., amongst others.
8. The isolated nucleic acid molecule according to claim 2 wherein the cellulose synthase or catalytic subunit thereof is the *Arabidopsis thaliana* RSW1 polypeptide.

- 171 -

9. The isolated nucleic acid molecule according to any one of claims 1 to 8, comprising a sequence of nucleotides which is at least 40% identical to any one of SEQ ID NOs:1, 3, 4, 5, 7, 9, 11 or 13 or a complementary sequence thereof.

5

10. The isolated nucleic acid molecule according to claim 9, wherein the percentage identity to any one of SEQ ID NOs:1, 3, 4, 5, 7, 9, 11 or 13 or a complementary sequence thereof is at least 60%.

10 11. The isolated nucleic acid molecule according to claim 9, wherein the percentage identity to any one of SEQ ID NOs:1, 3, 4, 5, 7, 9, 11 or 13 or a complementary sequence thereof is at least 80%.

12. An isolated nucleic acid molecule which comprises a sequence of nucleotides substantially as set forth in any one of SEQ ID NOs:3, 4, 5, 7, 9 or 11 or a homologue, analogue or derivative thereof or a complementary sequence thereto.

13. The isolated nucleic acid molecule according to any one of claims 1 to 12 , wherein said nucleic acid molecule hybridizes under at least low stringency conditions to at least 20 contiguous nucleotides of any one of SEQ ID NOs:1, 3, 4, 5, 7, 9, 11 or 13 or a complementary sequence thereto.

14. An isolated nucleic acid molecule which encodes a polypeptide which is capable of cellulose and/or  $\beta$ -1,4- glucan biosynthesis in a plant cell, fungal cell, insect cell, animal cell, yeast cell or bacterial cell when expressed therein.

15. The isolated nucleic acid molecule according to claim 14, wherein the polypeptide is cellulose synthase or a catalytic subunit thereof.

30 16. The isolated nucleic acid molecule according to claim 14 or 15, derived from a

prokaryote.

17. The isolated nucleic acid molecule according to claim 16, wherein the prokaryote is a bacterium other than *Agrobacterium tumefaciens*, *Acetobacter pasteurianus* or *Acetobacter xylinum*.

18. The isolated nucleic acid molecule according to claim 14 or 15, derived from a eukaryote.

10 19. The isolated nucleic acid molecule according to claim 18, wherein the eukaryote is a plant or fungus.

20. The isolated nucleic acid molecule according to claim 19, wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* 15 (rice), wheat, barley, maize, *Brassica* spp., *Eucalyptus* spp., hemp, jute, flax, *Pinus* spp., *Populus* spp., and *Picea* spp., amongst others.

21. The isolated nucleic acid molecule according to claim 20, wherein the cellulose synthase or catalytic subunit thereof is the *Arabidopsis thaliana* RSW1 polypeptide.

20

22. The isolated nucleic acid molecule according to any one of claims 14 to 21, comprising a sequence of nucleotides which is at least 40% identical to any one of SEQ ID NOs:1, 3, 4, 5, 7, 9, 11 or 13 or a complementary sequence thereto.

25 23. The isolated nucleic acid molecule according to claim 22, wherein the percentage identity to any one of SEQ ID NOs:1, 3, 4, 5, 7, 9, 11 or 13 or a complementary sequence thereof is at least 60%.

24. The isolated nucleic acid molecule according to claim 22, wherein the percentage 30 identity to any one of SEQ ID NOs:1, 3, 4, 5, 7, 9, 11 or 13 or a complementary sequence

thereof is at least 80%.

25. The isolated nucleic acid molecule according to claim 22, comprising the sequence of nucleotides substantially as set forth in any one of SEQ ID NOs:3, 4, 5, 7, 9 or 11 or a homologue, analogue or derivative thereof or a complementary sequence thereto.
26. An isolated nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes a polypeptide capable of cellulose and/or  $\beta$ -1,4-glucan biosynthesis wherein said polypeptide comprises a sequence of amino acids which is at least 10 40% identical to any one of SEQ ID Nos:2, 6, 8, 10, 12 or 14.
27. The isolated nucleic acid molecule according to claim 26, wherein the percentage identity to any one of SEQ ID Nos:2, 6, 8, 10, 12 or 14 is at least 60%.
- 15 28. The isolated nucleic acid molecule according to claim 27, wherein the percentage identity to any one of SEQ ID Nos:2, 6, 8, 10, 12 or 14 is at least 80%.
29. The isolated nucleic acid molecule according to claim 26, wherein the polypeptide comprises a sequence of amino acids substantially as set forth in any one of SEQ ID Nos:2, 20 6, 8, 10, 12 or 14.
30. A genetic construct which comprises the isolated nucleic acid molecule according to any one of claims 1 to 29.
- 25 31. A genetic construct which comprises the isolated nucleic acid molecule according to any one of claims 1 to 29 operably connected to a promoter sequence.
32. The genetic construct according to claim 31, wherein the nucleic acid molecule is operably connected to the promoter sequence in the sense orientation such that RNA which 30 encodes a polypeptide capable of cellulose and/or  $\beta$ -1,4-glucan biosynthesis or a homologue,

analogue or derivative thereof is produced when said nucleic acid molecule is expressed.

33. The genetic construct according to claim 31, wherein the nucleic acid molecule is operably connected to the promoter sequence in the antisense orientation such that RNA 5 which is complementary to RNA which encodes a polypeptide capable of cellulose and/or  $\beta$ -1,4-glucan biosynthesis or a homologue, analogue or derivative thereof, is produced when said nucleic acid molecule is expressed.

34. The genetic construct according to claim 33, wherein the nucleic acid molecule 10 encodes an antisense or ribozyme molecule.

35. The genetic construct according to any one of claims 31 to 34, wherein the promoter is the CaMV 35S promoter.

15 36. The genetic construct according to any one of claims 31 to 34, wherein the promoter is the *Arabidopsis thaliana RSW1* gene promoter.

37. A method of increasing the level of cellulose in a cell, tissue, organ or organism, said method comprising expressing the isolated nucleic acid molecule according to any one of 20 claims 1 to 29 therein, in the sense orientation, for a time and under conditions at least sufficient to produce or increase expression of the polypeptide encoded therefor.

38. The method according to claim 37, comprising the additional first step of transforming the cell, tissue, organ or organism with the isolated nucleic acid molecule.

25

39. The method according to claim 38, wherein the cell is a prokaryotic cell.

40. The method according to claim 38, wherein the cell, tissue, organ or organism is a eukaryotic cell, tissue, organ or organism.

30

- 175 -

41. The method according to claim 40, wherein the cell, tissue, organ or organism is a plant, fungal, insect, animal or yeast cell, tissue, organ or organism.
42. The method according to claim 41, wherein the cell, tissue, organ or organism is a 5 plant cell, tissue, organ or organism.
43. The method according to claim 42 wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants 10 such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.
44. A method of reducing the level of non-crystalline  $\beta$ -1,4-glucan in a cell, tissue, organ or organism, said method comprising expressing the isolated nucleic acid molecule according to any one of claims 1 to 29 therein, in the sense orientation, for a time and under conditions 15 at least sufficient to produce or increase expression of the polypeptide encoded therefor.
45. The method according to claim 44, comprising the additional first step of transforming the cell, tissue, organ or organism with the isolated nucleic acid molecule.
- 20
46. The method according to claim 44, wherein the cell is a prokaryotic cell.
47. The method according to claim 44, wherein the cell, tissue, organ or organism is a eukaryotic cell, tissue, organ or organism.
- 25
48. The method according to claim 47, wherein the cell, tissue, organ or organism is a plant, fungal, insect, animal or yeast cell, tissue, organ or organism.
49. The method according to claim 48, wherein the cell, tissue, organ or organism is a 30 plant cell, tissue, organ or organism.

50. The method according to claim 50 wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

5

51. A method of reducing the level of starch in a cell, tissue, organ or organism, said method comprising expressing the isolated nucleic acid molecule according to any one of claims 1 to 29 therein, in the sense orientation, for a time and under conditions at least sufficient to produce or increase expression of the polypeptide encoded therefor.

10

52. The method according to claim 50, comprising the additional first step of transforming the cell, tissue, organ or organism with the isolated nucleic acid molecule.

53. The method according to claim 51, wherein the cell is a prokaryotic cell.

15

54. The method according to claim 53, wherein the cell, tissue, organ or organism is a eukaryotic cell, tissue, organ or organism.

55. The method according to claim 54, wherein the eukaryote is a plant, fungus, insect, animal or yeast.

56. The method according to claim 55, wherein the eukaryote is a plant.

57. The method according to claim 56 wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

58. A method of reducing the level of cellulose in a cell, tissue, organ or organism, said method comprising expressing the isolated nucleic acid molecule according to any one of

claims 1 to 29 therein, in the antisense orientation, for a time and under conditions at least sufficient to prevent or reduce the expression of the polypeptide encoded therefor.

59. The method according to claim 58, comprising the additional first step of  
5 transforming the cell, tissue, organ or organism with the isolated nucleic acid molecule.

60. The method according to claims 58 or 59, wherein the cell, tissue, organ or organism  
is a eukaryotic cell, tissue, organ or organism.

10 61. The method according to claim 60, wherein the eukaryote is a plant, fungus, insect,  
animal or yeast.

62. The method according to claim 61, wherein the eukaryote is a plant.

15 63. The method according to claim 62 wherein the plant is selected from the list  
comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice),  
*Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants  
such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

20 64. A method of increasing the level of non-crystalline  $\beta$ -1,4-glucan in a cell, tissue,  
organ or organism, said method comprising expressing the isolated nucleic acid molecule  
according to any one of claims 1 to 29 therein, in the antisense orientation, for a time and  
under conditions at least sufficient to prevent or reduce the expression of the polypeptide  
encoded therefor.

25

65. The method according to claim 64, comprising the additional first step of  
transforming the cell, tissue, organ or organism with the isolated nucleic acid molecule.

30 66. The method according to claims 64 or 65, wherein the cell, tissue, organ or organism  
is a eukaryotic cell, tissue, organ or organism.

- 178 -

67. The method according to claim 66, wherein the eukaryote is a plant, fungus, insect, animal or yeast.

68. The method according to claim 67, wherein the eukaryote is a plant.

5

69. The method according to claim 68 wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

10

70. A method of increasing the level of starch in a cell, tissue, organ or organism, said method comprising expressing the isolated nucleic acid molecule according to any one of claims 1 to 29 therein, in the antisense orientation, for a time and under conditions at least sufficient to prevent or reduce the expression of the polypeptide encoded therefor.

15

71. The method according to claim 70, comprising the additional first step of transforming the cell, tissue, organ or organism with the isolated nucleic acid molecule.

72. The method according to claims 70 or 71, wherein the cell, tissue, organ or organism  
20 is a eukaryotic cell, tissue, organ or organism.

73. The method according to claim 72, wherein the eukaryote is a plant, fungus, insect, animal or yeast.

25 74. The method according to claim 73, wherein the eukaryote is a plant.

75. The method according to claim 74 wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants  
30 such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

76. A method of producing a recombinant enzymatically active polypeptide which is capable of synthesizing cellulose and/or  $\beta$ -1,4-glucan and/or an intermediate between cellulose and  $\beta$ -1,4-glucan in a cell, said method comprising expressing the isolated nucleic acid molecule according to any one of claims 1 to 29 or a homologue, analogue or derivative thereof in said cell for a time and under conditions sufficient for the polypeptide encoded therefor to be produced.

77. The method according to claim 76, comprising the additional first step of transforming the cell with the isolated nucleic acid molecule according to any one of claims 1 to 29 or the genetic construct according to any one of claims 11 to 15.

78. A recombinant polypeptide produced according to the method defined by claim 76 or 77.

79. The recombinant cellulose biosynthetic polypeptide according to claim 78, further defined as a recombinant cellulose synthase or catalytically active subunit thereof.

80. A recombinant cellulose biosynthetic polypeptide capable of cellulose and/or  $\beta$ -1,4-glucan production and comprising a sequence of amino acids set forth in any one of SEQ ID Nos: 2, 6, 8, 10, 12 or 14 or a homologue, analogue or derivative thereof which is at least 40% identical thereto.

81. The recombinant cellulose biosynthetic polypeptide according to claim 80, wherein the percentage identity to any one of SEQ ID Nos: 2, 6, 8, 10, 12 or 14 is at least 60%.

25

82. The recombinant cellulose biosynthetic polypeptide according to claim 81, wherein the percentage identity to any one of SEQ ID Nos: 2, 6, 8, 10, 12 or 14 is at least 80%.

83. The recombinant cellulose biosynthetic polypeptide according to claim 82, comprising a sequence of amino acids substantially as set forth in any one of SEQ ID Nos: 2, 6, 8, 10,

- 180 -

12 or 14.

84. A method of altering the mechanical properties of a cell wall, said method comprising expressing the isolated nucleic acid molecule according to any one of claims 1 to 29 in the 5 antisense orientation in said cell for a time and under conditions sufficient for the level of non-crystalline  $\beta$ -1,4-glucan to increase in said cell.

85. The method according to claim 84 wherein the non-crystalline  $\beta$ -1,4-glucan is cross-linked to cellulose microfibrils.

10

86. The method according to claim 84 or 85 wherein the cell wall normally has a high ratio of cellulose to hemicelluloses.

15

87. The method according to any one of claims 84 to 86, wherein the nucleic acid molecule expressed in the antisense orientation is contained within an antisense molecule or ribozyme molecule.

88. The method according to any one of claims 84 to 87, wherein the cell wall is a plant cell wall.

20

89. The method according to claim 88, wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

25

90. An antibody molecule which binds to the recombinant polypeptide according to any one of claims 78 to 83 or a homologue, analogue or derivative thereof.

30

91. A transgenic plant transformed with the isolated nucleic acid molecule according to any one of claims 1 to 29 or a genetic construct according to any one of claims 30 to 36.

92. The transgenic plant according to claim 91, wherein said plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

5

93. Use of an isolated nucleic acid molecule according to any one of claims 1 to 29 to modify the cellulose content of a cell.

94. Use according to claim 93, wherein if the nucleic acid molecule according to any one of claims 1 to 29 is expressed in the sense orientation in said cell, the level of cellulose therein is increased.

95. Use according to claim 93, wherein if the nucleic acid molecule according to any one of claims 1 to 29 is expressed in the antisense orientation in said cell, the level of cellulose therein is decreased.

96. Use according to claim 95, wherein said cell is further characterised by increased non-crystalline  $\beta$ -1,4-glucan content and/or starch content.

20 97. Use according to claim 95 or 96, wherein said cell is further characterised by increased cross-linking of non-crystalline  $\beta$ -1,4-glucan to cellulose.

98. Use according to any one of claims 93 to 97, wherein the cell is a plant cell.

25 99. Use according to claim 98 wherein the plant is selected from the list comprising *Arabidopsis thaliana*, *Gossypium hirsutum* (cotton), *Oryza sativa* (rice), *Eucalyptus* spp., *Brassica* spp., wheat, barley, maize, hemp, jute, flax, and woody plants such as *Pinus* spp., *Populus* spp., *Picea* spp., amongst others.

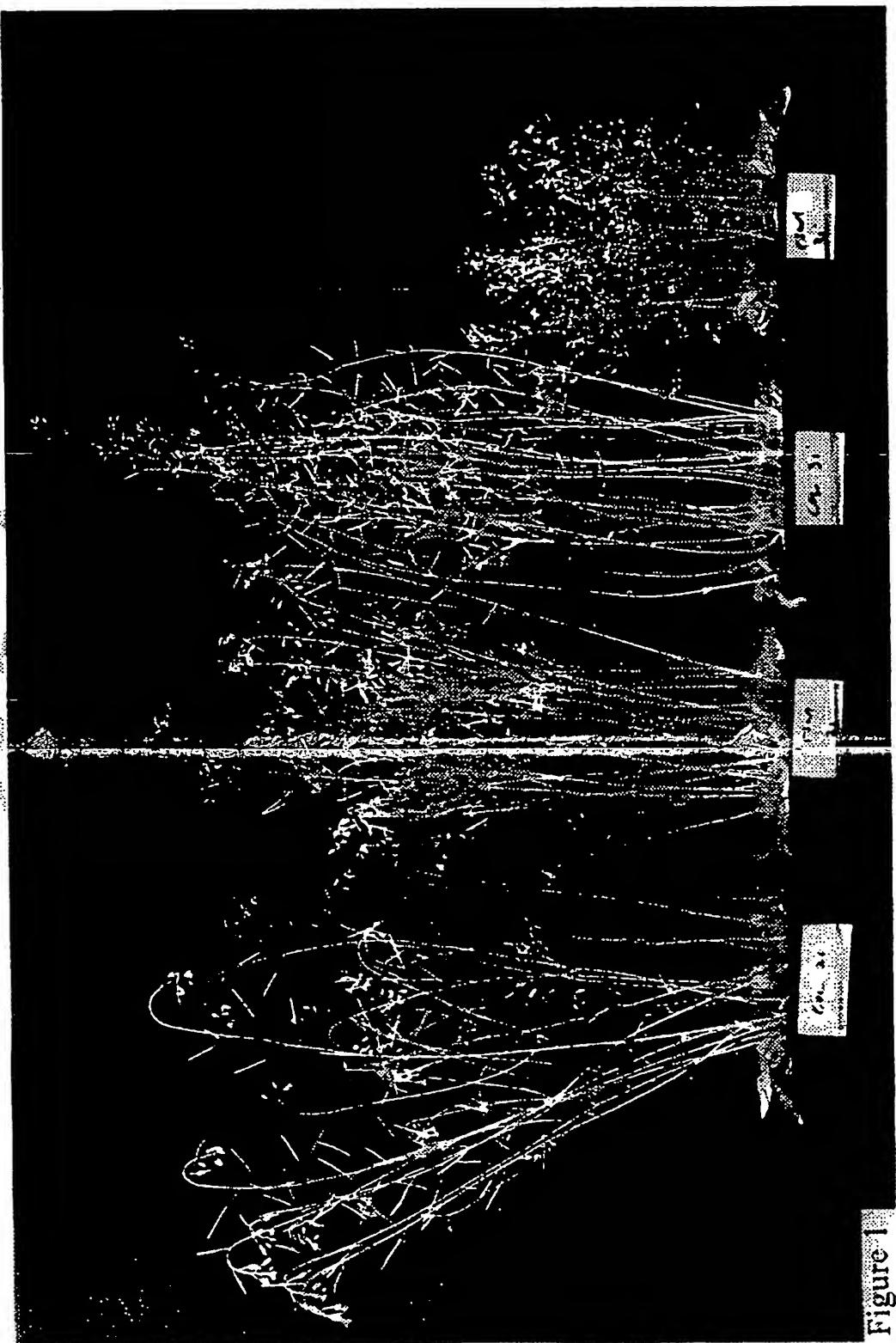


Figure 1

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2/21

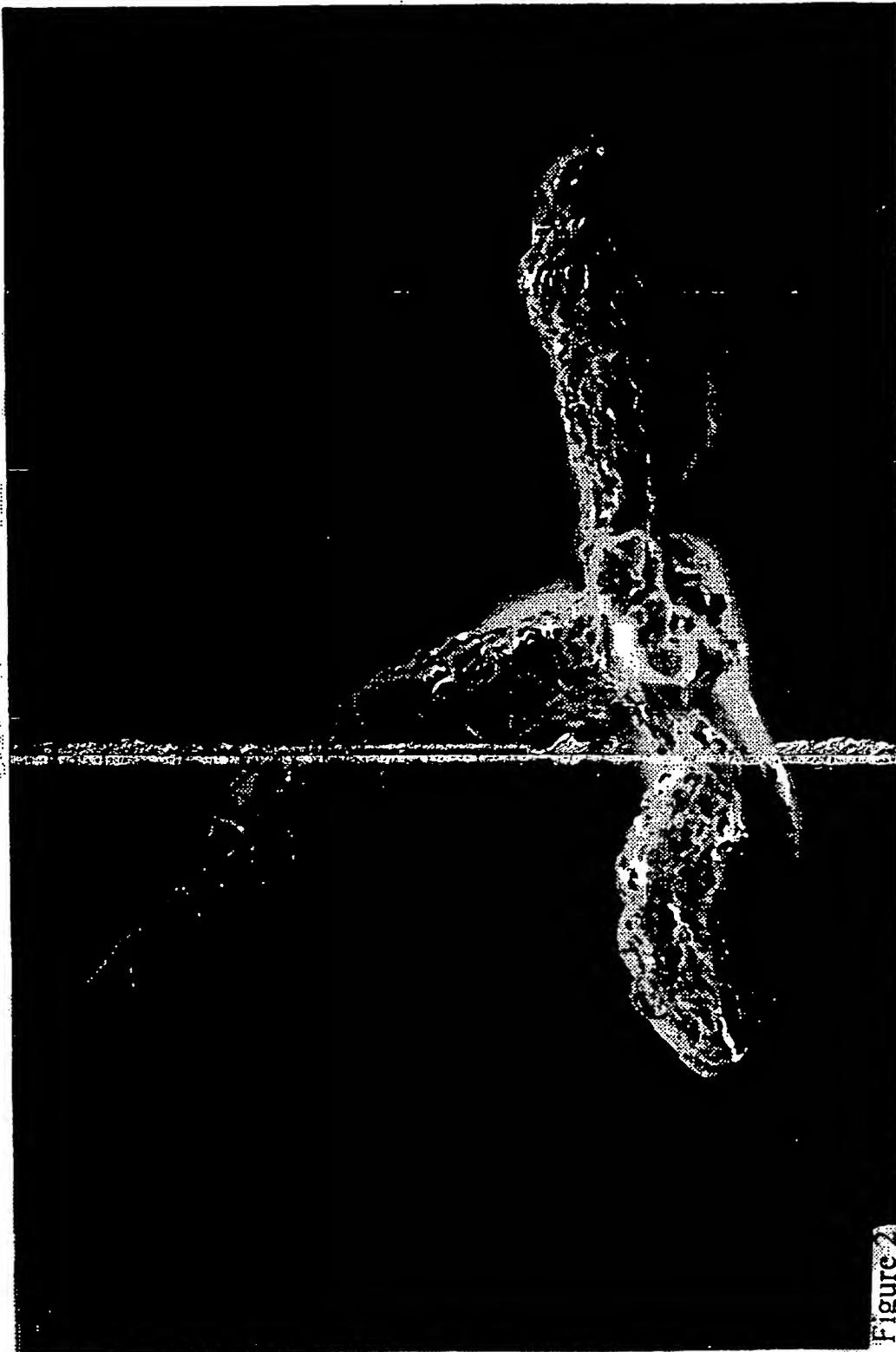


Figure 2

3/21

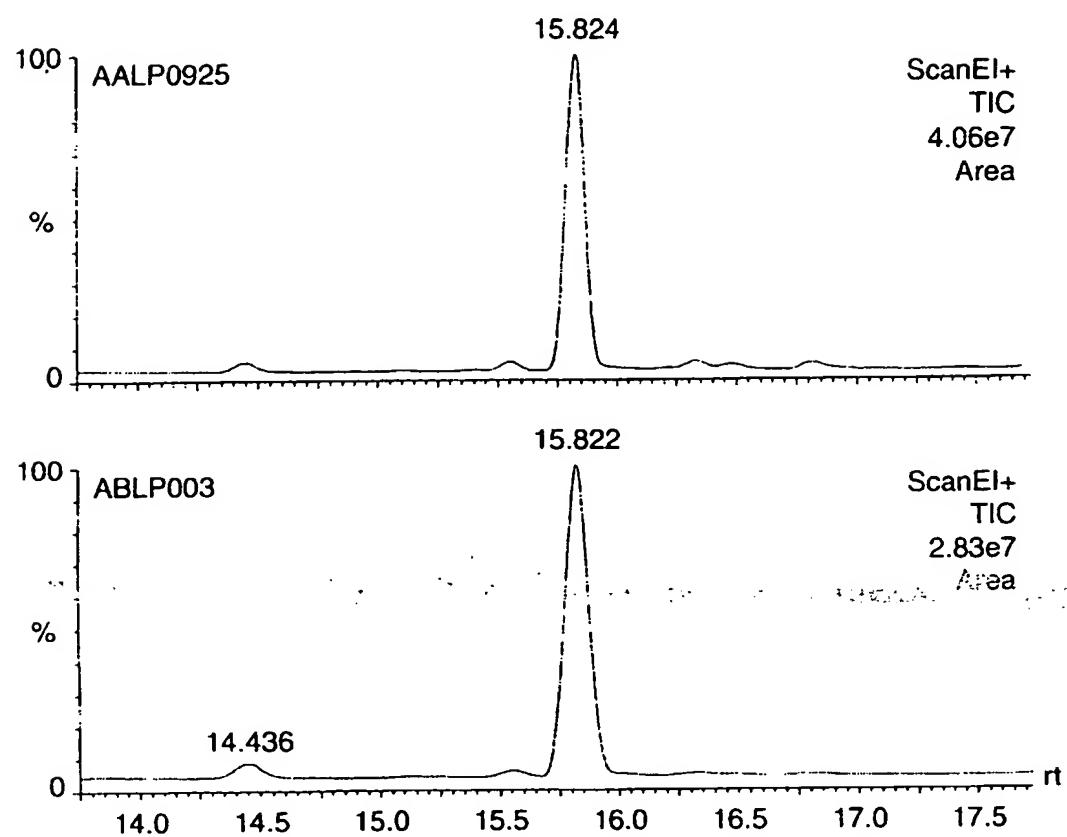


Figure 3

4/21

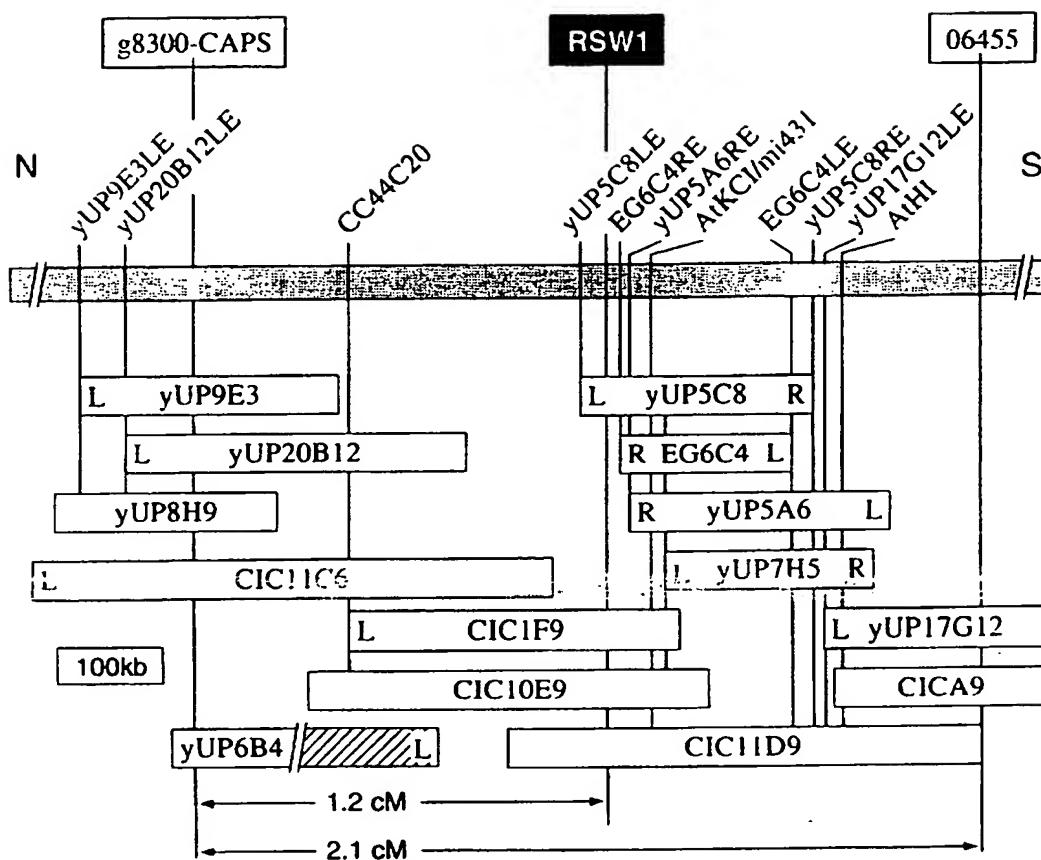


Figure 4

5/21

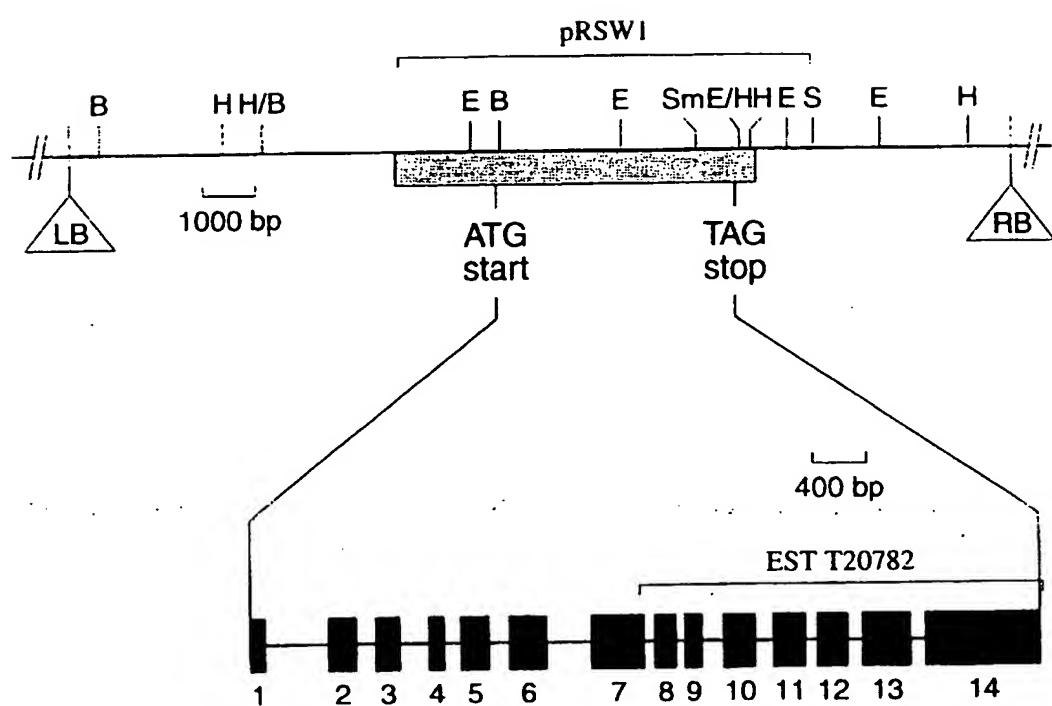


Figure 5

6/21

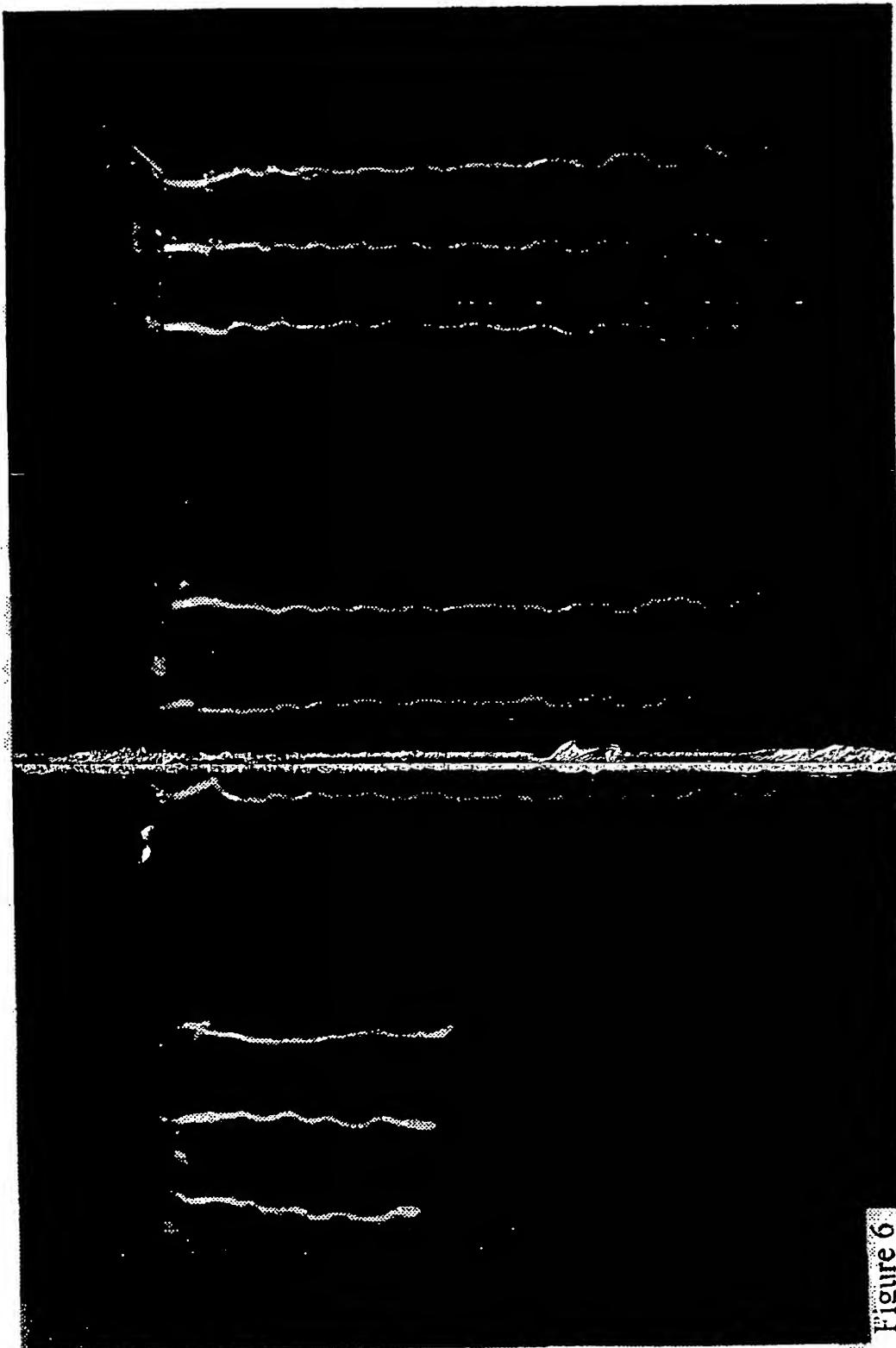


Figure 6

7/21

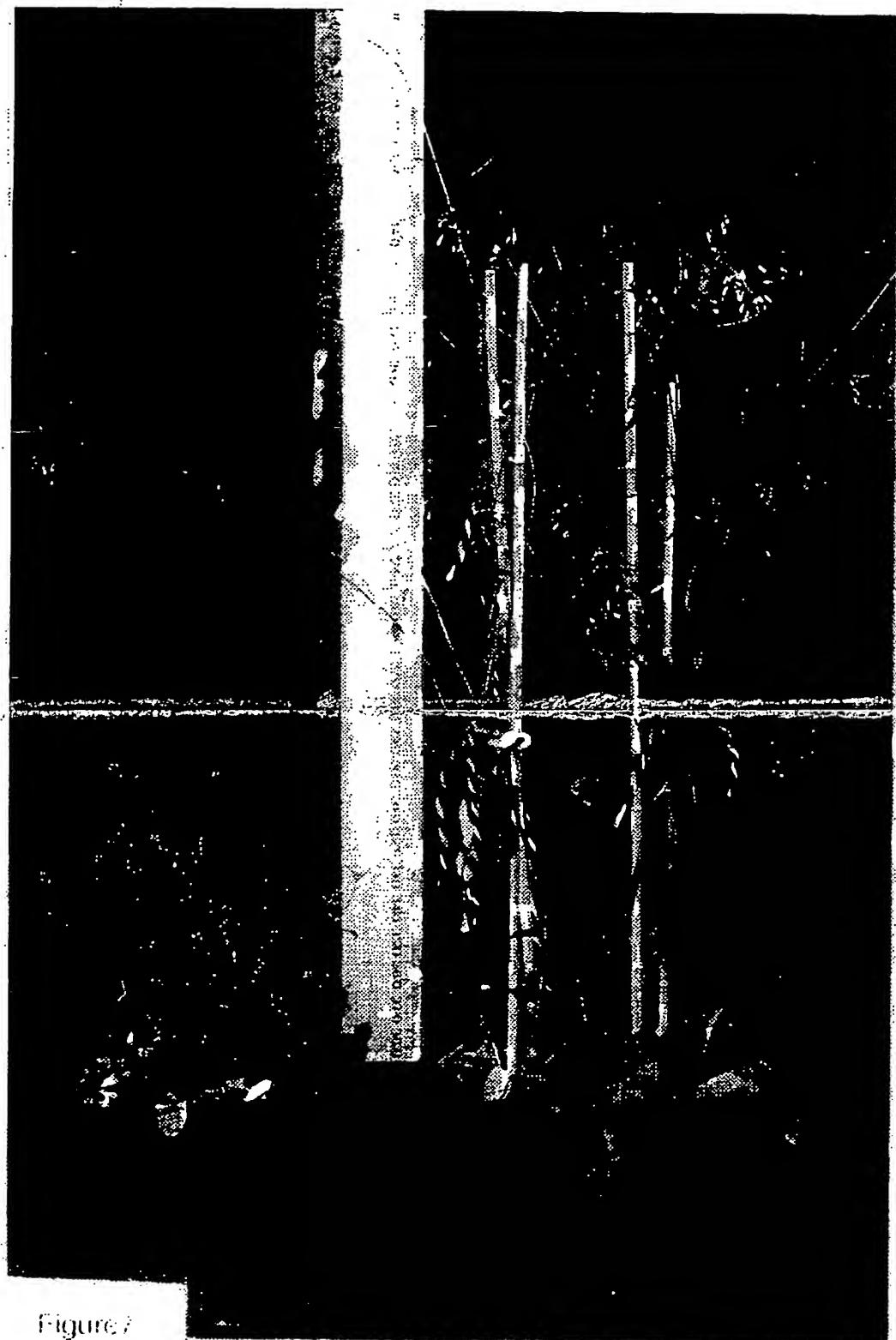


Figure 7

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8/21

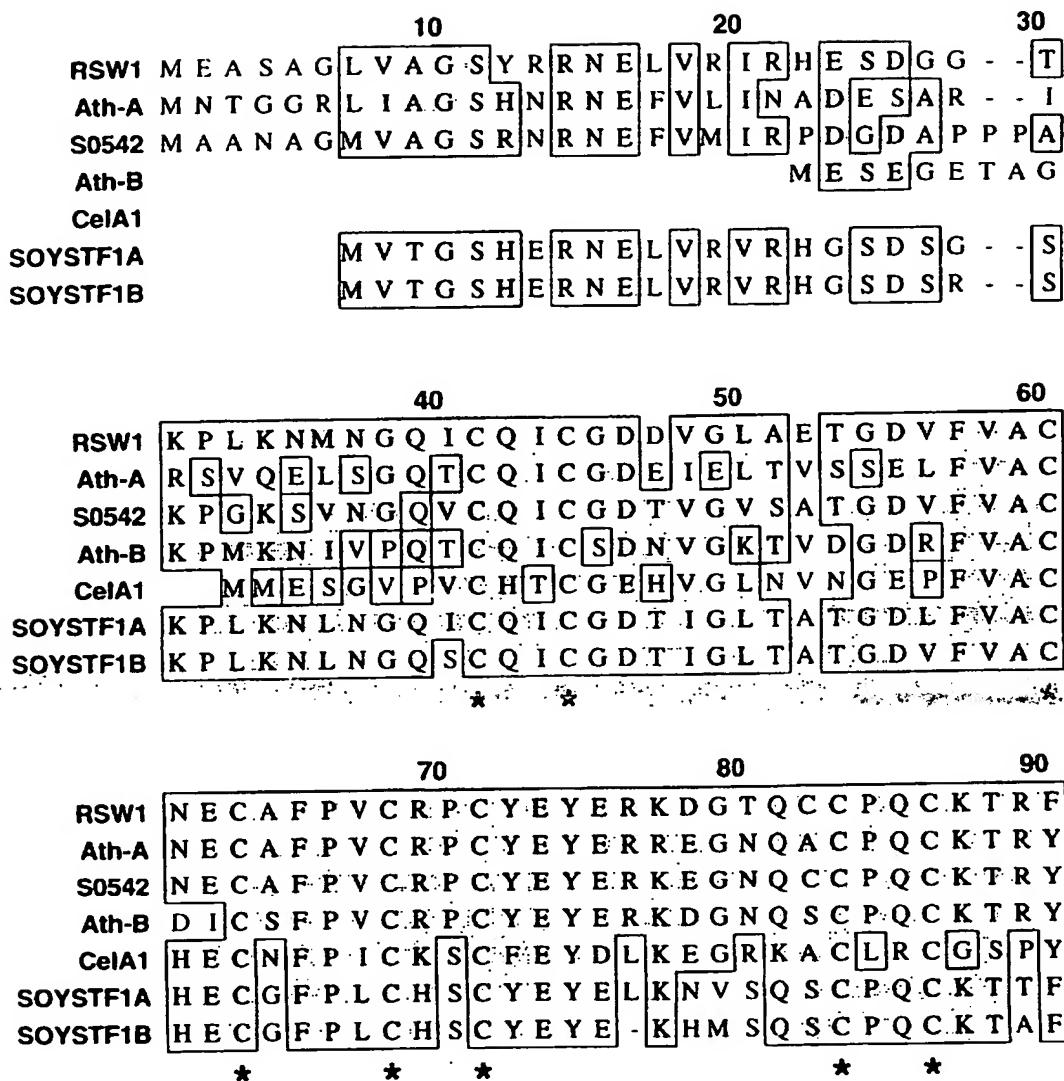


Figure 8

9/21

Cont I
Cont II
Cont II
Cont IV
Cont V
Cont VI
Cont VII
Cont VIII
Cont IX
Cont X

10/21

	10	20	30	40	50	60
<b>R5W1</b>	MEASAGLVAGSYRRNELVRIRHESDGG.					
<b>Ath-A</b>	MNTGGRILLAGSHNRNEFVLLINADESAR.	. IRSVQELSGOTCQICGDEIELTVSSELFVAC				
<b>S0542</b>	MAANAGMVAGSRNRFVMIRPDKDAPPAPAKPGKSVNGQVCQICGDTVGVSATGDVFVAC					
<b>Ath-B</b>	MESEGETAGKPMKNIVPQTCQICSDNVGKTVDGDRFVAC					
<b>CelA1</b>	MMESGVPVCHTCGEHVGLNVNGEPEPFVAC					
<b>CelA2</b>						
<b>D48636</b>						
	70	80	90	100	110	120
<b>R5W1</b>	NECAFPPVCRPCYEEVERKDGTQCCPQCKTRFRRHIRGSPRVEGDEDEDVDDIENEFNYAQG					
<b>Ath-A</b>	NECAFPPVCRPCYEEVERREGNQACPQCKTRYKR1KGSPRVDGDDEEEEDIDDLEYEFDHGM					
<b>S0542</b>	NECAFPPVCRPCYEEVERKEGNQCCPQCKTRYKRHKGCPRVQGDDEEEEDVDDLDNEFHYKHG					
<b>Ath-B</b>	DICSFPPVCRPCYEEVERKDGNQS CPQCKTRYKR1KGSPAI PGDKDEDGLADEGTVEFNYPQ					
<b>CelA1</b>	HECNFPICKSCFNEYDLKEGRKAICLRCGSPPYDENILDDVEKATGDQSTMMAHLINKSQDVGI					
<b>CelA2</b>						
<b>D48636</b>						

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**FIGURE 9 (CONT 1)**

11/21

RSW1	ANKA	130	140	150	160	170	180
Ath-A	DPEHAAEAALSSRLNTGRGGIISAPPGSQIPLLTYCDEDADMYSDRHIALIVPPSTGYGNR	.....	.....	.....	.....	.....	.....
S0542	NGKGPEWQI	.....	.....	.....	.....	.....	.....
Ath-B	K.EKISERMLGWHLTRGKGEEMGEPOYDKEVSHNHLPRLTSRQDTSGEFSAAASPERLSVS	.....	.....	.....	.....	.....	.....
Cel-A1	.....	.....	.....	.....	.....	.....	.....
Cel-A2	.....	.....	.....	.....	.....	.....	.....
<b>D48636</b>							
RSW1	SGPLGPSDRNAIASSPYIDPRQFVPVVRIVDPSKDLNSYGLGNVDWKERVEGWKLKQEKNML	190	200	210	220	230	240
Ath-A	PAPFTDSSAPPQARSMSVPQKDIAEYGYGSVAWKRDRMEVWKRRQGEKLQ	.....	.....	.....	.....	.....	.....
S0542	TSSYVDPSVPVPVVRIVDPSKDLNSYGINSVWDQERVAWRNKQDKNM	.....	.....	.....	.....	.....	.....
Ath-B	IAGGKRLPYSQSPNRRIVDPVGFLGNVAWKERVDGWMKQEKNTG	.....	.....	.....	.....	.....	.....
Cel-A1	HARHTSSVSTLDSEMAEDNGNSIWKNRVESWKEKRNKKK	.....	.....	.....	.....	.....	.....
Cel-A2	.....	.....	.....	.....	.....	.....	.....
<b>D48636</b>	STRPGNVAWKERVDGWMKIKDKGAT						

FIGURE 9 (CONT III)

12/21

<b>RSW1</b>	QMT . . . . .	250	260	270	280	290	300
<b>Ath-A</b>	VIK . . . . .						
<b>S0542</b>	HEGGNNGRGSNDDDELDDPDMMPMMDEGRQPLSRKLIPIRSSRINPYRMLILC						
<b>Ath-B</b>	QVA . . . . .						
<b>PV</b>	NKYYPEARGGDMEGTGSNGEDIQMVDARLPLSRIVPIPSSRNQLNLRYRIVIL						
<b>Cel-A1</b>	PAT . . . . .						
<b>Cel-A2</b>	STQAASERGGVDIDASTDILADEALLNDEARQLLSRKVSIPSSRNIPYRMVIML						
<b>D48636</b>	PAT . . . . .						
	TKVEREAEIPPEQQMEDKPADPDAQPLSTIPIPKSRLAPIRTVIMV						
	PMTNNGTSIAPSEGRGVGDIIDAST'DYNMEDALLNDETRQPLSSRKVPLPSSRNIPYRMVIVL						
<b>RSP1</b>	310	320	330	340	350	360	
<b>Ath-A</b>	RLIILGLFFQYRTTHPVKNAYPLWLTSVICEIWFAFSWILDQFPKWKYPINRETYLDRLAI						
<b>S0542</b>	RLAILGLFFHYRILHPVNDAYGLWLTSVICEIWFAVSVICELDQFPKWKYPIERETYLDRLSL						
<b>Ath-B</b>	RLIIILMFFFOYRVTHPVVRDAYGLWLVSVICEIWILPLSWILDQFPKWKYPINRETYLDRLAL						
<b>Cel-A1</b>	RLVILCLFLHYRITNPVPNAFALWLVSVICEIWFAWSWILDQFPKWKFPVNRETYLDRLAL						
<b>Cel-A2</b>	RLIIILGLFFHYRVTNPVDSAFGLWLTSVICEIWFAFSWILDQFPKWKYPVNRETYLDRLSA						
<b>D48636</b>	RLVVVLISIFLHYRITNPVRNAYPLWLLSERVICEIWFAWSWILDQFPKWFPINRETYLDRLAL						

**FIGURE 9 (CONT III)**

13/21

	370	380	390	400	410	420
<b>RSSW1</b>	RYDRDGEPSQLVPPVDFVSTVDPLKEPPLVTANTVLSILSVDDYPVDKVACYVSSDDGSAML					
<b>Ath-A</b>	RYEKEGKPSGLAPVDFVSTVDPLKEPPLITANTVLSILAVDYPVDKVACYVSSDDGSAML					
<b>S0542</b>	RYDREGEPSQLAPIDVFFVSTVDPLKEPPLITANTVLSILAVDYPVDKVSCYVSSDDGSAML					
<b>Ath-B</b>	RLVILCLFLHYRITNPVPNAFAIWLVSVICEIWFAWSILDQFPKWFPPVNRETYLDRLL					
<b>Cel-A1</b>	RYEREGEPDELAADVDFFVSTVDPLKEPPLITANTVLSILALDYPVDKVSCYISDDGSAML					
<b>Cel-A2</b>						
<b>D48636</b>	RYDRGEPSQLAAVDIFVSTVDPMKEPPLVTANTVLSILAVDYPVDKVSCYVSSDDGSAML					
	430	440	450	460	470	480
<b>RSSW1</b>	TFESSLSETAEFAKKWVPFCKKFNIEPRAPEFYFAAQKIDYLKDKIQPSFVKERRAMKREYE					
<b>Ath-A</b>	TFEALSDTAEFARKWVPFCKKFNIEPRAPEFYFAAQKIDYLKDKIQPSFVKERRAMKRDYE					
<b>S0542</b>	TFEALSETAEFARKWVPFCKKHNIEPRAPEFYFAAQKIDYLKDKIQPSFVKERRAMKREYE					
<b>Ath-B</b>	SFESLAETSEFARKWVPFCKKSIEPRAPEWFYFAAKIDYLKDKVQTSFVKDRRAMKREYE					
<b>Cel-A1</b>	TFESILVETADFARKWVPFCKKSIEPRAPEFYFSQKIDYLKDKVQPSFVKERRAMKRDYE					
<b>Cel-A2</b>	RRWVVPFCKKHNVPRAPEFYFNEKIDYLKDKVHPSFVKERRAMKREYE					
<b>D48636</b>	TFDALAETSEFARKWVPFVKYNNIEPRAPEWFYSQKIDYLKDKVHPSFVKDRRAMKREYE					

FIGURE 9 (CONT IV)

14/21

<b>R5W1</b>	490	500	510	520	530	540
<b>Ath-A</b>	EFKVRINALVAKAQKIPPEEGWTMODGTPWPGNNTRDHPGMIQVFLGHSGGLDTDGNELPR					
<b>S0542</b>	EFKVRINALVAKAQKVPEEEGWTMQDGTWPWGNNVRDHPGMIQVFLGHSGVRDTDGNELPR					
<b>Ath-B</b>	EFKVRINALVAKAQKVPEEEGWTIMADGTAWPGNNP RDHPGMIQVFLGHSGGLDTDGNELPR					
<b>Cel-A1</b>	EFKIRINALVAKCPEEEGWTMQDGTWPWGNNPRDHPGMIQVFLGQNGGLDAEGNELPR					
<b>Cel-A2</b>	EYKIRINALVAKAQKTPDEGWIMQDGTSWPGNNPRDHPGMIQVFLGYSGARDIEGNELPR					
<b>D48636</b>	EFKVRINALVAKAQKKPEEEGWTMQDGTWPWGNNTRDHPGMIQVYLGSAGALDVDGKELPR					
<b>R5W1</b>	550	560	570	580	590	600
<b>Ath-A</b>	LIYVSREKRPGFQHKKAGAMNALIRVSAVLTNGAYLLNNVDCDHYFNNSKAIKEAMCFMM					
<b>S0542</b>	LIYVSREKRPGFDHKKAGAMNSLIRVSAVLNSNAPYLLNVDCHYINNSKAIRESMCFMM					
<b>Ath-B</b>	LVYVSREKRPGFQHKKAGAMNALIRVSAVLTNGPFILNLDCDHYINNSKALREAMCFLM					
<b>Cel-A1</b>	LVYVSREKRPGYQHKKAGAENALVRVSAVLTNAFPILNLDCDHYVNNSKAVREAMCFLM					
<b>Cel-A2</b>	LVYVSREKRPGYQHKKAGAENALVRVSAVLTNAFPILNLDCDHYINNSKAMREAMCFLM					
<b>D48636</b>	LVYVSREKRPGFQHKKAGAMNALVRVSAVLTNGQYMLNLDCDHYINNSKALREAMCFLM					

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FIGURE 9 (CONT V)

15/21

<b>RSW1</b>	610	620	630	640	650	660
<b>Ath-A</b>	DPAIGKKCCYYVQFPQRFDGIDLHDRYANRNTIVFFDINMKGLDGIQGPVYVGTGCCFNRQA					
<b>S0542</b>	DPQSGKKVCYVQFPQRFDGIDRHDYNSRNNTVFFDINMKGLDGIQGPVYVGTGVFRKQA					
<b>Ath-B</b>	DPNLGKQVCYVQFPQRFDGIDKNDRYANRNTVFFDINLRGLDGIQGPVYVGTGVFNRTA					
<b>Cel-A1</b>	DPQVGRDVCYVQFPQRFDGIDRSIDRSDRYANRNTVFFDINMKGLDGIQGPVYVGTGVFNRTA					
<b>Cel-A2</b>	DPQFGGKKLCLYYVQFPQRFDGIDRHDYANRNTVFFDINMGLDGIQGPVYVGTGVFNRTA					
<b>D48636</b>	DPNLGRSVCYVQFPQRFDGIDRNDRYANRNTVFFDINLRGLDGIQGPVYVGTGVFNRTA					
<b>RSW1</b>	670	680	690	700	710	720
<b>Ath-A</b>	LYGYDPVLTEDLEPNIIVKSCCGSRKKSSKKYNYE.....					
<b>S0542</b>	LYGFDAPKKKPPGKTCNCWPWKCCLCGGLRKKSKTKA.....					
<b>Ath-B</b>	LYYEPIKVHKKPSSLSKLCGGSRKKNSKAKKESDK.....					
<b>Cel-A1</b>	LYGYGPPSMSFPKSSS.....SSCSCCCPGKKEPKDPS.....					
<b>Cel-A2</b>	LYGYDPPVSEKRPKMTDCWPSWCCCCGGSRKKSKKGEKKGLLYGKKMMGKN.....					
<b>D48636</b>	LYYEPIKQKKGSFLSSLLCGGRKKASKSKKKSSDK.....					

**FIGURE 9 (CONT VI)**

16/21

<b>R SW1</b>	730	740	750	760	770	780
<b>Ath-A</b>	GINRSDSNAPLFNMDIDEFGFEGYDDERSILMSQRSVEKRGOSPVFIATAATEMEQGGIIPP					
<b>S0542</b>	NTKETSKQIHALENVDEGVIVPVSNEKERSEATQLKLEKKFGQSPFVASVLQNGGVPR					
<b>Ath-B</b>	HTDS.TVPVFNLDDIEEGVEGAGFDDEKALLMSQMSLEKRGQSAVFVASTLMEENGGVPP					
<b>Cel-A1</b>	KREELDAALFLNLREIDN. . . YDEYEYRSMMLISQTSFEKTFGLSSVFIESTLMEENGVAE					
<b>Cel-A2</b>	YVKKGSAAPVFDLEEIEEGILEG. YEELEKSTLMSQKNFEKRGQSPFVIASLMEENGGLPE					
<b>D48636</b>	HVDS.AVPVFNLLEDIEEGVEGAGFDDEKSLLMSQMSLEKRGQSAAFVASTLMEYGGVPO					
<b>R SW1</b>	790	800	810	820	830	840
<b>Ath-A</b>	TTNPATLLKEAIHVVISCGYEDKTEWGKEIGWIYGSVTEDILTGFKMHARGWRSIYCNPPR					
<b>S0542</b>	NASPACLLREAIQVISCGYEDKTEWGKEIGWIYGSVTEDILTGFKMHCIGWRSSVYCMPKR					
<b>Ath-B</b>	SATPENFLKEAIHVVISCGYEDKSDWGMEIGWIYGSVTEDILTGFKMHARGWRSIYCMPLK					
<b>Cel-A1</b>	SANPSTLIKEAIHVVISCGYEEKTAWGKEIGWIYGSVTEDILTGFKMHCIGWRSSVYCMPLR					
<b>Cel-A2</b>	GTNSTSLIKEAIHVVISCGYEEKTEWGKEIGWIYGSVTEDILTGFKMHCIGWRSSVYCVPKR					
<b>D48636</b>	SATPESLLKEAIHVVISCGYEDKTEWGTEIGWIYGSVTEDILTGFKMHARGWRSIYCMPLK					

FIGURE 9 (CONT VIII)

17/21

<b>R5W1</b>	PAFKGSAPINLSDRLNQVLRWA LGSSIEILLSSRHCPIWYGYHG.	850	860	870	880	890	900
<b>Ath-A</b>	AAFKGSAPINLSDRLHQLRWA LGSSVEIFLSRHCPIWYGYGG.						
<b>S0542</b>							
<b>Ath-B</b>	PAFKGSAPINLSDRLNQVLRWA LGSSVEIFLSRHCPIWYGYNG.						
<b>Cel-A1</b>	PAFKGSAPINLSDRLHQVLRWA LGSSVEIFLSRHCPLWYGYGG.						
<b>Cel-A2</b>	PAFKGSAPINLSDRLHQVLRWA LGSSVEIFLSRHCPLWYGYGG.						
<b>D48636</b>	PAFKGSAPINLSDRLNQVLRWA LGSSVEIFLSRHCPIWYGYGG.						
<b>R5W1</b>	ITSIPLIAYCILPAFCLITDRF IPIPEISNYASIW	910	920	930	940	950	960
<b>Ath-A</b>	WTSLPLIVYCSLPAVCLL TGKEIVPEISNYAGILFMLM						
<b>S0542</b>							
<b>Ath-B</b>	ITSIPLLMYCTLLAVCLFT TNQFIIPQISNIASIWF						
<b>Cel-A1</b>	FTSPLLIAYCSLPAILT GKFIIPTLMSNLASVLF						
<b>Cel-A2</b>	FTSPLLIAYCTIPAVCLL TGKFIIPTLMSNLTSVWF						
<b>D48636</b>	LTSIPLLIYCVLPAILT GKFIIPEISNFASIWF						

**FIGURE 9 (CONT VIII)**

18/21

<b>R5W1</b>	WRNEQFWVIGGTSAAHLFAVFOGLIKVLAGIDTNFTVTSKATDEDGDFAEELYIFKWTTLLI	970	980	990	1000	1010	1020
<b>Ath-A</b>	WRNEQFWVIGGASSHLFQGCLLKVLAGVNNTNFTVTSKAAD . DGAFSELYIFKWTTLLI						
<b>S0542</b>							
<b>Ath-B</b>	WRNEQFWVIGGVSA AHLFAVFOGLILKVL AGIDTNFTVT SKASDEDGDFAEELYLFKWTTLLI						
<b>Cel-A1</b>	WRNEQFWVIGGVSA AHLFAVFOGFLIKMLAGIDTNFTVTAKAAD . DADFGELYIVKWTTLLI						
<b>Cel-A2</b>	WRNEQFWVIGGVSA AHLFAVFOGLIKVLAGVDTNFTVTAKAAD . DTEFGELYLFKWTTLLI						
<b>D48636</b>	WRNEQFWVIGGI SAH LFAVFOGLILKVL AGIDTNFTVT SKASDEDGDFAEELYMFKWTTLLI						
<b>R5W1</b>	PPTTVLVLNLIGIVAGVSYAVNSGYQSWGPLFGKLFFALWVIAHLYPFLKGLLGRQNRT P	1030	1040	1050	1060	1070	1080
<b>Ath-A</b>	PPTTLLIINIIVGVS DAI SNGYD SWGPLFGCRLLFFALWVIVHLYPFLKGMLGKDMP						
<b>S0542</b>							
<b>Ath-B</b>	PPTTLLIVNLVGVVAGVSYA INSGYQSWGPLFGKLFFFAFWVIVHLYPFLKGLMGRQNRT P						
<b>Cel-A1</b>	PPTTLLIVNMVGVVAGFS DALNKGYEA WGPLFGKVFFSFWVILHLYPFLKGLMGRQNRT P						
<b>Cel-A2</b>	PPTTLLIILNMVGVVAGVSDA INNGYGSWGPLFGKLFFFAFWVIVHLYPFLKGLMGRQNRT P						
<b>D48636</b>	PPTTLLIINLVGVVAGISY A INSGYQSWGPLFGKLFFFAFWVIVHLYPFLKGLMGRQNRT P						

FIGURE 9 (CONT IX)

19/21

	1090	1100	1110	1120
<b>RSS1</b>	TIVIVWSVLLASIFSLWVRINPFDANPNANNFNGKGGVF			
<b>Ath-A</b>	TIVVWSVLLASILLLLWVRINPEVAK.	GGPVLEICGLNCGN		
<b>S0542</b>				
<b>Ath-B</b>	TIVVVWSVLLASIFSLWVRIDPFTSRVTGPDILECGINC			
<b>Cel-A1</b>	TIVVLWSVLLASVFSLVWVRINPFDVSTADSTTVSQSCISIDC			
<b>Cel-A2</b>	TIVVLWSVLLASIFSLWVRIDPFLPKQTGPVLUKQCGVEC			
<b>D48636</b>	TIVVWAIIASIFSLWVRIDPFTTRVTGPDITQTCGGINC			

FIGURE 9 (CONT X)

20/21

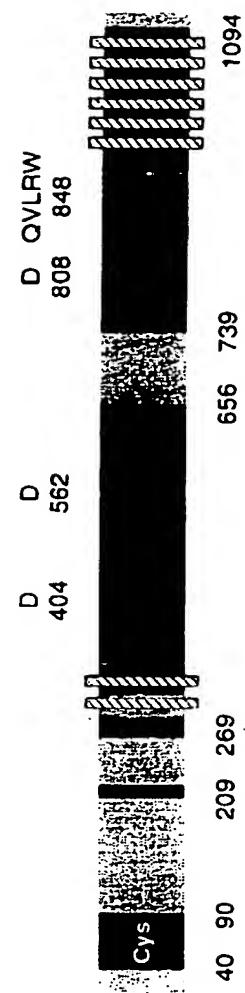


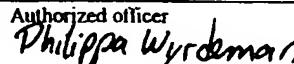
FIGURE 10

21/21

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00402

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int Cl <sup>6</sup> : C12N 15/54, 9/10		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) See Electronic Database Box below		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See Electronic Database Box below		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPAT, Medline, ChemAbs, Genebank, Swiss Prot, EMBL Search Terms: Cellulose Biosynthesis, Cellulose Synthase, Sequence ID# 2.		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91/13988 (THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM), 19 September 1991 see whole document	1-4, 14-16, 30-32
X	WO 92/18631 (WEYERHAESUR COMPANY) 29 October 1992 see whole document	1-4, 14-16
X	WO 90/12093 (CETUS CORPORATION) 18 October 1990 see whole document	1-4, 14-16
<input type="checkbox"/> Further documents are listed in the continuation of Box C		<input checked="" type="checkbox"/> See patent family annex
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search  14 August 1997		Date of mailing of the international search report  <b>18 AUG 1997</b>
Name and mailing address of the ISA/AU <b>AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION</b> PO BOX 200 WODEN ACT 2606 AUSTRALIA      Facsimile No.: (06) 285 3929		Authorized officer  for JIM CHAN Telephone No.: (06) 283 2340

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00402

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International Application No.  
PCT/AU 97/00402

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	9113988	AU	75569/91				
WO	9012098	AU	54373/90	CA	2014264	EP	471687
		IL	94053	NZ	233312	US	5268274
WO	9218631	US	5268274	NZ	233312	CA	2014264
		IL	94053	AU	54373/90	EP	471687

END OF ANNEX